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SPOTTING OF FIGS ON THE MARKET [1]

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INTRODUCTION

Figs (*Ficus carica* L.) that are poorly refrigerated in transit or that are held on the market after arrival are likely to develop a surface spotting that seriously detracts from their market value. This spotting occurs on fruit from the Atlantic as well as on that from the Pacific coast and on all varieties that have been under observation. Smith and Hansen [3] have described a disease that appears to be identical with that under consideration and have reported its occurrence in the San Joaquin Valley of California both on the fruit on the tree and on fruit in transit to the cannery. They found *Alternaria* sp. to be the causal organism.

DESCRIPTION AND CAUSE OF THE DISEASE

The spotting is confined largely to fully ripe fruit and is favored by cracks in the skin and by the sugary solution often found on the surface of the fig. Species of *Alternaria*, *Cladosporium*, and *Botrytis* have been isolated from the diseased areas, but *Alternaria* has been the prevailing fungus.

ALTERNARIA

The *Alternaria* spots are first evident as small grayish-white tufts of mycelium; as these enlarge they soon darken to an olivaceous color [4] (fig. 1). At first the fungus appears to have but little attachment to the skin and sometimes can be rubbed off with little evidence of injury; but as the spots enlarge and become slightly sunken, any attempt to remove the fungus also removes the skin. The organism apparently can penetrate the skin readily. The mycelium is soon found in abundance at a depth of several cells with scattering hyphae much deeper in the tissue (fig. 2).

The foregoing description applies particularly to the *Alternaria* spotting on California figs. Figs from Norfolk, Va., have a similar spotting due to *Alternaria*, but the fungus retains its whitish-gray color longer and never becomes as dark as the California fungus (fig. 3).

The two forms of *Alternaria* also show differences in growth on culture media (figs. 4, 5, 6, 7). In Petri-dish cultures the Virginia *Alternaria*, unlike the California form, often develops a radial appearance and a hairy, threadlike growth, and on water agar it shows a tendency to coil (fig. 7).

[1] Received for publication August 26, 1937; issued May 1938.

[2] The authors are indebted to W. T. Pentzer and C. E. Asbury, of the Division of Fruit and Vegetable Crops and Diseases, for cooperation in obtaining the figs from Fresno, Calif., and to C. O. Bratley, of the same Division, for cooperation in obtaining those purchased on the New York market.

[3] SMITH, R. E., and HANSEN, H. N. FRUIT SPOILAGE DISEASES OF FIGS. Calif. Agr. Expt. Sta. Bull. 506, 84 pp., illus. 1931.

[4] This and later color references are based on Ridgway standards. RIDGWAY, R. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 pp., illus. Washington, D. C. 1912.

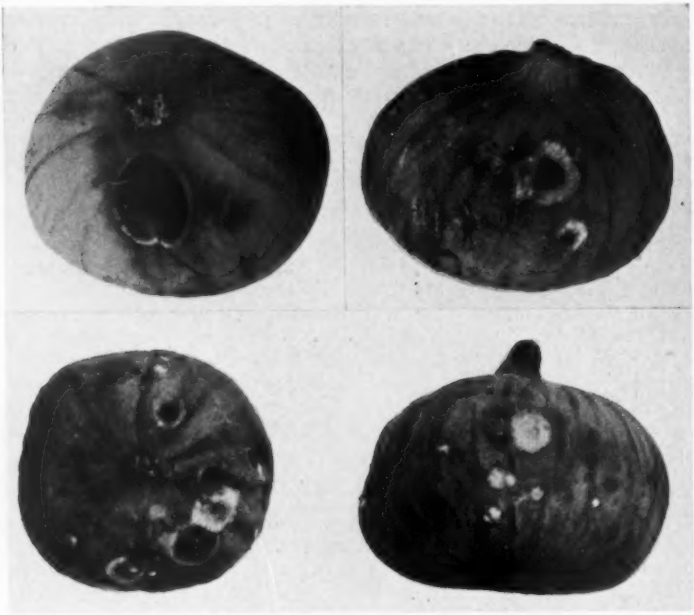

FIGURE 1.—*Alternaria tenuis* on Calimyrna figs as received from California.

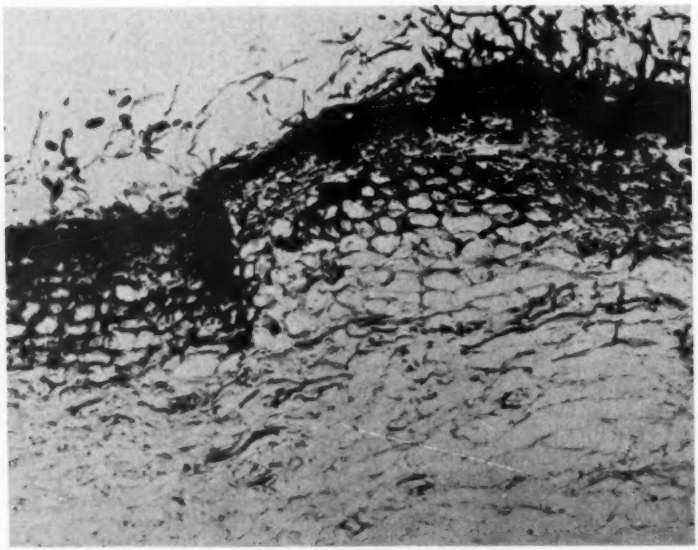

FIGURE 2.—Photomicrograph of an *Alternaria* lesion from a California Black Mission fig as seen in radial section. × 140. Note the spores and mycelium on the surface, the thick network of hyphae between the outer layers of cells, and the occasional hypha at a depth of 15 or 20 cells.

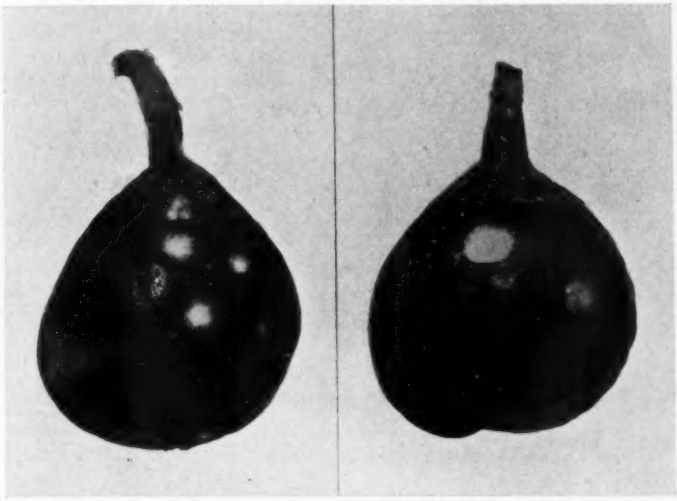

FIGURE 3.—Spotting on California Black Mission figs resulting from inoculation with the *Alternaria* isolated from Norfolk, Va., figs.

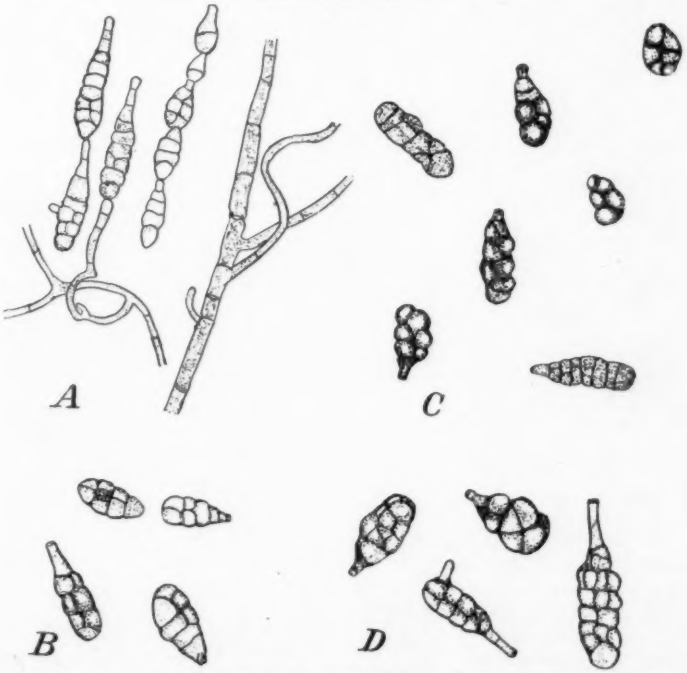

FIGURE 4.—Conidia of the California *Alternaria*. *A*, From a 30-day culture on water agar; *B*, from California figs; *C*, secondary characters of conidia from a culture on potato-dextrose agar; *D*, secondary characters of conidia from an inoculation on figs. × 450.

The following measurements and studies are based largely on water-agar cultures. The scant mycelial development on this medium gives an excellent opportunity to follow the spore formation, and the spores and sporophores are apparently typical.

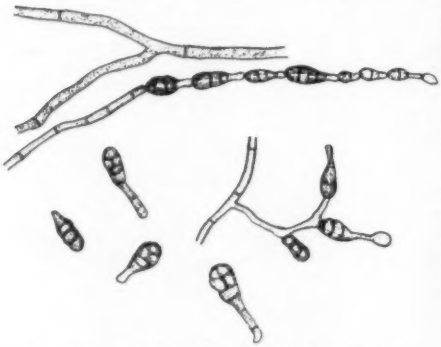

FIGURE 5.—Conidia of the Virginia *Alternaria* from a culture on water agar. × 450.

The conidiophores are darker than the mycelium, and they may be either erect or decumbent. On the California *Alternaria*, they were 25μ to 250μ by 3μ to 5μ and were sometimes moderately echinulate; on the Virginia *Alternaria*, they were 20μ to 180μ by 4μ to 5μ and echinulation was not observed.

The conidia of the Virginia form were produced in short chains, having usually three to four and sometimes as many as nine conidia to a chain. They were obclavate to pyriform, muriform; sometimes echinulate; at first pale yellow green but later olivaceous; one- to five-septate; and measured 17μ to 35μ by 6μ to 10μ.

The conidia of the California form were produced in long chains that often developed secondary branching. The conidia were obclavate to pyriform and broadly muriform; definitely echinulate; at first pale to amber yellow but later olivaceous to fuscous; usually short-beaked; longitudinally and transversely septate; and measured 20μ to 75μ by 8μ to 17μ. The development of secondary character was found on all substrata tested, the conidia finally became much distorted and swollen, and septa formed at various angles.

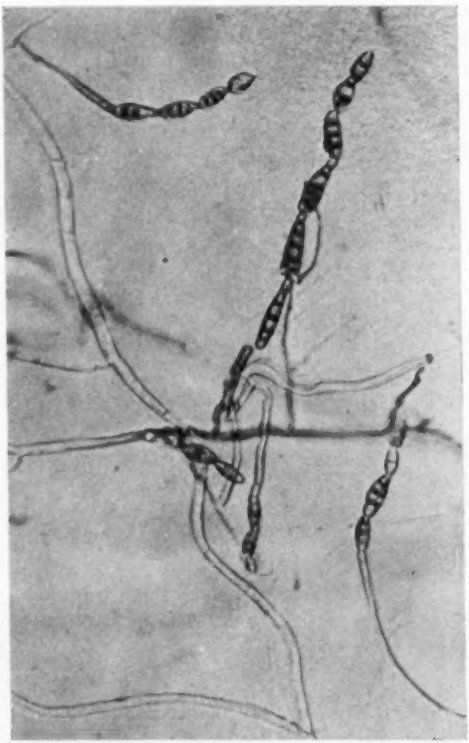

FIGURE 6.—Photomicrograph of the California *Alternaria* after 4 days on water agar. × 270.

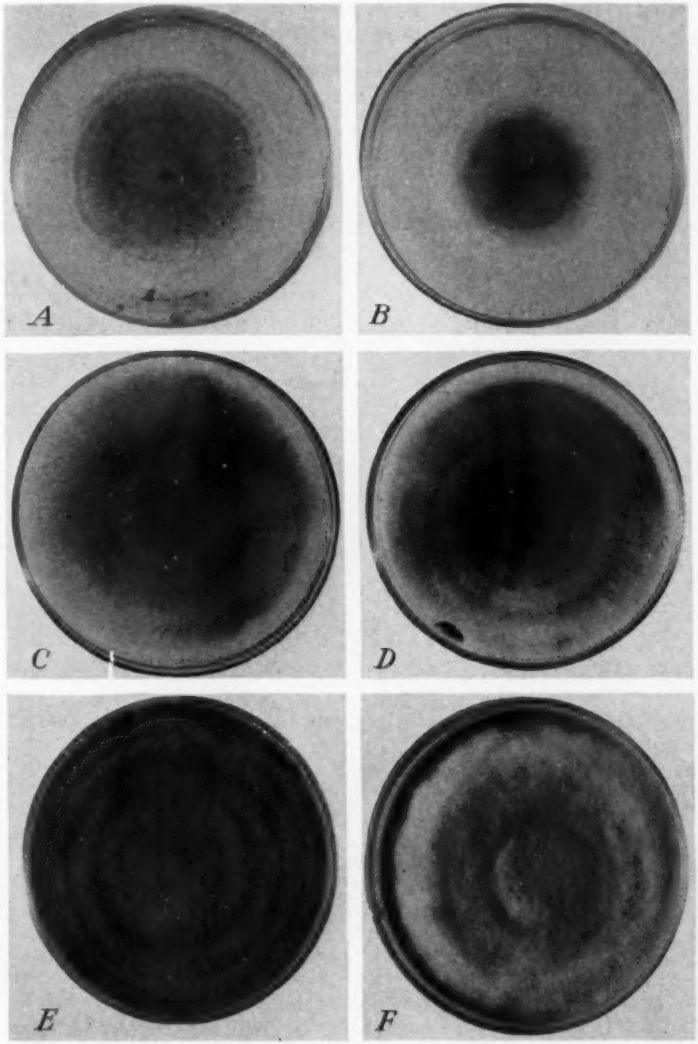

FIGURE 7.—Growth contrasts of the California *Alternaria* and the Virginia *Alternaria* after 8 days at room temperature: *A*, California *Alternaria* on water agar; *B*, Virginia *Alternaria* on water agar; *C*, California *Alternaria* on corn-meal agar; *D*, Virginia *Alternaria* on corn-meal agar; *E*, California *Alternaria* on fig agar; *F*, Virginia *Alternaria* on fig agar.

The California organism is readily included in the *Alternaria tenuis* group as described by Elliott,[5] and there does not seem to be sufficient ground for excluding the Virginia organism from this group. Until further studies of the genus are made it seems best to consider the two fungi as strains or varieties of *A. tenuis* Nees.

CLADOSPORIUM

A *Cladosporium* isolated from spots on California figs was found to have some pathological significance. Figure 8 shows the type of spotting produced on Calimyrna figs after inoculation with a spore suspension of this fungus.

The *Cladosporium* spots appear first as dark olive-green specks that are particularly noticeable on the light-skinned varieties. The

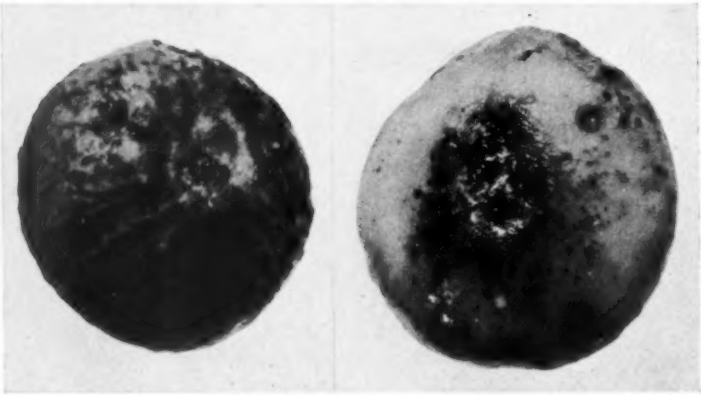

FIGURE 8.—*Cladosporium* spots on Calimyrna figs after inoculation with a spore suspension and prolonged holding at 32° F.

lesions enlarge, become slightly depressed, and turn to a yellowish-olive color.

Conidial production begins early, and the surface growth on the fruit is composed largely of conidiophores and conidia. The conidiophores arise in tufts, erect or nearly so, from a nodular mass of cells embedded in the epidermis. They are septate and brownish olive in color and are 3μ to 4.8μ in diameter.

The conidia take various forms—cylindrical, elliptical, ovoid, subfusiform, and subglobose. All are continuous at first but later the basal and immediately adjacent conidia may become once or more septate (fig. 9). Occasionally other conidia become once septate. As the conidia mature they change from hyaline to pale greenish yellow and finally become olivaceous. Excluding the elongated basal cells, the conidia measure 3μ to 7.2μ by 2μ to 5.6μ. The basal cells, which are readily detached as conidia, measure 12μ to 24μ by 3.6μ to 5μ. The conidial forms in culture were similar to those found on the fruit.

[5] ELLIOTT, J. A. TAXONOMIC CHARACTERISTICS OF THE GENERA ALTERNARIA AND MACROSPORIUM. Amer. Jour. Bot. 4: 439-476, illus. 1917.

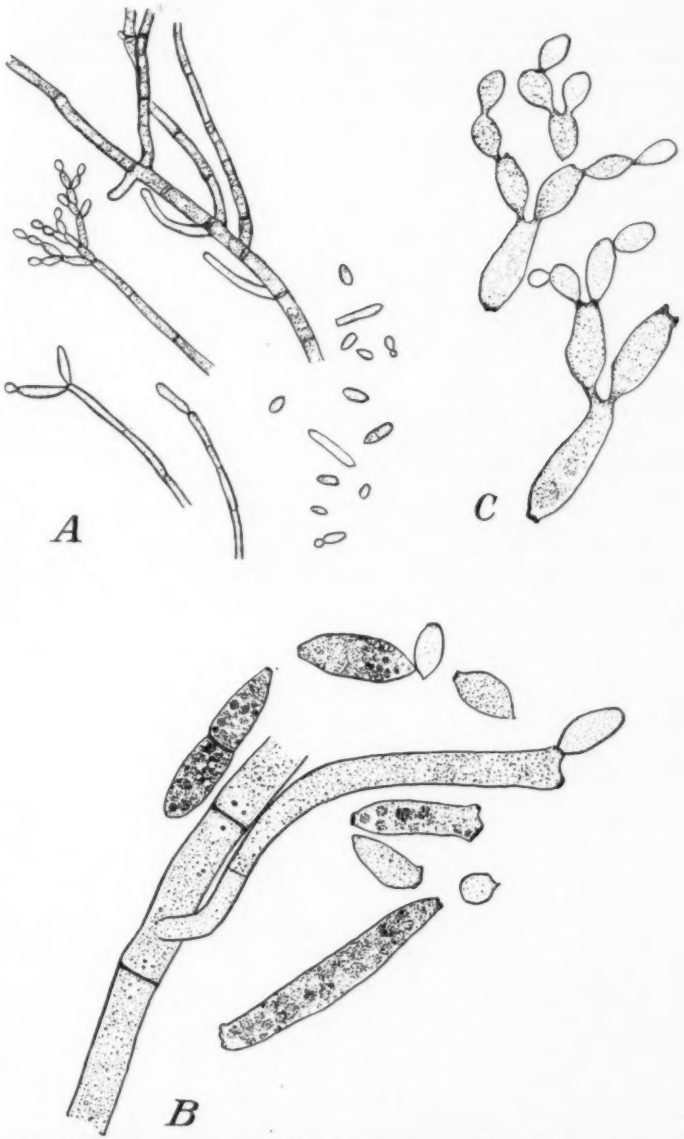

FIGURE 9.—Conidia and conidiophores of *Cladosporium herbarum* isolated from California figs. *A*, on Thaxter agar. ×450. Note the branching or arbor type of spore formation. *B*, On water agar. ×1,700. Showing details of conidial forms. *C*, On water agar. ×1,700. Part of a conidial chain showing dichotomous branching.

On figs and on agar cultures, the branching of the conidial arbor was predominantly dichotomous rather than sympodial, and the conidial formation was of the *Hormodendron* type. However, the comparative studies of Brooks and Hansford,[6] Bennett,[7] and Robak[8] place this *Hormodendron* as a form or variety of *Cladosporium herbarum* (Pers.) Link. The writers have compared the fungus under consideration with a culture of *Cladosporium herbarum* received from Dr. Johnanna Westerdijk, director of Centraalbureau voor Schimmelcultures, Baarn, Netherlands, and have found no significant difference between the two organisms. The fungus is therefore classified as *C. herbarum* (Pers.) Link.

While this *Cladosporium* has been isolated from figs and has produced spots when inoculated on the figs, it should be emphasized that it is not of great economic importance. Its occurrence has been confined largely to overripe fruit. The spotting of otherwise marketable figs is caused almost entirely by *Alternaria*.

BOTRYTIS

Botrytis sp. was occasionally found on overripe figs, but the fungus was inclined to spread over the surface of the fruit in a manner that set it entirely apart from the spotting under consideration.

MATERIALS AND METHODS

The fruit used in the present experiments was obtained from various sources, and the following lots will be referred to later by number. The variety names for the eastern lots are those given by the salesmen or the producers.

Lot 1.—Black Mission figs that had been held in storage at Exeter, Calif., for 15 days, July 1932, were the source of the original cultures of *Alternaria*.

Lot 2.—Kadota, Black Mission, Black Spanish, and Calimyrna figs were picked at Fresno, Calif., August 8, 1932, and forwarded to Yakima, Wash., by ordinary express. They arrived in Yakima August 10, free from mildew and in good condition, and were used in experiments the following day.

Lot 3.—Black Mission figs from Sultana, Calif., purchased on the Washington, D. C., market July 13, 1933.

Lot 4.—Black Spanish figs from Norfolk, Va., purchased on the Washington, D. C., market August 17, 1933.

Lot 5.—Calimyrna figs from California, purchased on the New York market August 25, 1933, and forwarded to Washington, D. C., by ordinary express.

Lot 6.—Brown Turkey figs from Norfolk, Va., purchased on the Washington, D. C., market September 6, 1933.

Lot 7.—Figs purchased from a local grower, Washington, D. C., September 11, 1933: (a) Black Spanish or related variety; (b) Green Ischia or related variety.

Lot 8.—Brown Turkey figs from Norfolk, Va., purchased on the Washington, D. C., market September 27, 1933.

Lot 9.—Black Mission figs from California, purchased on the Washington, D. C., market June 6, 1934.

Lot 10.—Black Mission figs from California, purchased on the New York market June 30, 1934, and taken to Washington, D. C., the same day.

Lot 11.—Calimyrna figs, purchased on the New York market August 9, 1934, and taken to Washington, D. C., the same day.

Lot 12.—Calimyrna figs, purchased on the New York market August 13, 1934, and forwarded to Washington, D. C., by ordinary express.

[6] BROOKS, F. T., and HANSFORD, C. G. MOULD GROWTHS UPON COLD-STORE MEAT. Brit. Mycol. Soc. Trans. 8: 113–142, illus. 1923.

[7] BENNETT, F. T. ON CLADOSPORIUM HERBARUM: THE QUESTION OF ITS PARASITISM, AND ITS RELATION TO "THINNING OUT" AND "DEAF EARS" IN WHEAT. Ann. Appl. Biol. 15: 191–213, illus. 1928.

[8] ROBAK, H. INVESTIGATIONS REGARDING FUNGI ON NORWEGIAN GROUND WOOD PULP AND FUNGAL INVASION AT WOOD PULP MILLS. Nyt. Mag. Naturvidenskab. 71: 185–330, illus. 1932.

Lot 13.—Calimyrna figs, purchased on the New York market August 20, 1934, and forwarded to Washington, D. C., by ordinary express.

Lot 14.—Calimyrna figs, purchased on the New York market August 17, 1935, and taken to Washington, D. C., the same day.

Lot 15.—Calimyrna figs, purchased on the New York market August 21, 1935, and forwarded to Washington, D. C., by ordinary express.

Lot 16.—Calimyrna figs, purchased on the New York market September 3, 1935, and forwarded to Washington, D. C., by ordinary express.

The inoculations were made in all cases by spraying the figs with sterile-water spore suspensions of organisms previously isolated from figs. After inoculation the figs were held in the open until dry before being placed under the experimental conditions. All of the figs were apparently free from spotting at the beginning of the experiments. In part of the tests, as mentioned later, the figs were disinfected with a bichloride of mercury wash prior to inoculation.

From 15 to 20 fruits were used under each condition in each experiment. Great care was used in selecting the figs so that those to be compared would be as nearly alike as possible in maturity and in freedom from injury.

Unless otherwise stated the spotting reported in the following experiments was of the type produced by *Alternaria tenuis*.

EFFECT OF HUMIDITY

Smith and Hansen[9] reported that fig spotting was likely to develop after summer rains or periods of high humidity and that at certain times during the season almost all figs of the white varieties would develop the disease if they were confined in a moist chamber.

TABLE 1.—*Effect of humidity on the development of spotting on figs*

Lot No.	Fungus	Temperature	Period of holding	Spots per fruit with—		
				High humidity [1]	Medium humidity [2]	Low humidity [3]
		° *F.*	*Days*	*Number*	*Number*	*Number*
3	*Alternaria*	59	4	31.8	5.8	
5	*Alternaria*	59	3	6.8	2.1	
8	*Alternaria*	59	3	1.6	.5	
9	*Alternaria*	50	2	1.9	1.1	
		40	3	1.3	.9	
		32	5	.8	.9	
10	*Alternaria*	50	2	1.6	1.4	
		40	2	.7	.3	
		32	4	3.4	2.2	
11	*Alternaria*	50	5	2.0	1.5	
		40	5	.6	.9	
		32	5	1.0	.8	
12	*Alternaria*	50	[4] 5	13.8	12.1	
		40	[4] 5	10.3	7.1	
		32	[4] 5	4.4	4.3	
13	*Alternaria*	50	[4] 5	3.1	2.9	
		40	[4] 5	1.4	1.1	
		32	[4] 5	1.9	1.8	
16	*Alternaria*	50	8	3.8	2.6	0
	Cladosporium	50	8	3.0	2.1	0

[1] Humidity of 90 percent or more.
[2] Humidity of 70 to 80 percent.
[3] Humidity of approximately 65 percent.
[4] During the last 3 of the 5 days all lots were at 50° F. and high humidity.

[9] SMITH, R. E., and HANSEN, H. N. See footnote 3.

In the present humidity studies the figs of lots 3, 5, and 8 were held in glass jars of 9-quart capacity and those of lot 16 and lots 9 to 13, inclusive, were held in metal boxes of approximately 1-cubic-yard capacity. Low humidity was maintained by the use of calcium chloride and high humidity by means of damp cloth or damp filter paper. With lot 3 the air was stagnant; with lots 5 and 8 it was slowly renewed; and with the remaining lots it was kept in motion by means of fans. The results of the experiments are shown in table 1.

All of the figs were inoculated, as previously described (p. 481.) The figs of lot 16 were disinfected with bichloride of mercury before inoculation; those of the other lots were not. An uninoculated lot at high humidity was included in most of the tests. The number of spots on the uninoculated figs was about one-fourth as great as that on the inoculated figs. The figs stored under medium humidity showed only slight shriveling, but it was evident in most cases that the moisture was as low as practicable for maintaining the product in a marketable condition. The figs held at low humidity (65 percent) soon became too badly withered to be marketable.

Storage at low humidity entirely prevented the development of spots. The figs held at medium humidity had less spotting in nearly every instance than those held at high humidity, but this reduction was seldom more than 30 percent. The effect of humidity appeared greater at 59° F. than at any of the lower temperatures. In the single test with *Cladosporium* the humidity effects were similar to those obtained with *Alternaria*.

The results show that humidity may be a factor of importance in the development of spotting, but they do not indicate that it is likely to form the basis for practical control.

EFFECT OF TEMPERATURE

The effect of temperature upon the development of fig spotting was tested both on fruit and on culture media.

Three series of experiments were made with the California *Alternaria* on culture media. In each series five Petri plates were used at each of the six temperatures. The curves of figure 10 give the average of the results of the different experiments

A study of the curves shows that the *Alternaria* growth rate was approximately twice as rapid at 77° as at 59° F., about twice as rapid at 68° as at 50°, nearly three times as rapid at 59° as at 41°, and fully three times as rapid at 41° as at 32°.

The effect of temperature upon the number of spots on the fruit is shown in table 2. In several of the tests the notes were taken after all of the lots had been held at a common temperature for 1 or more days after removal from the special temperatures. The results give a correct picture of what is likely to happen when figs are removed from storage, but do not show as great contrast as if the figs had been continued at the different storage temperatures until the time of note taking.

Notes taken at the time the figs were removed from the special storage conditions indicate that the effect of temperature on the number of *Alternaria* spots on the fruit is fully as great as the effect on the rate of growth in Petri dish cultures (fig. 10). In a single test with

the Virginia *Alternaria*, temperature contrasts were obtained similar to those reported in table 2 for the California *Alternaria*.

In the three tests with *Cladosporium* (table 2), the effect of temperature upon spotting was similar to that in the corresponding tests with *Alternaria*.

The spots at the higher temperatures were usually much larger than those at the lower temperatures as well as more numerous. There seems to be no doubt of the extreme value of low temperature in the prevention of spotting of figs.

EFFECT OF CARBON DIOXIDE

The effect of carbon dioxide upon *Alternaria* and *Cladosporium* was tested both on culture media and on the fruit. The storage atmos-

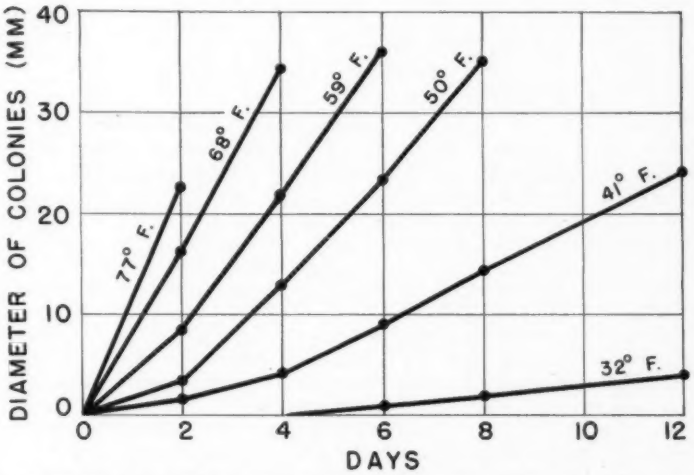

FIGURE 10.—Effect of temperature on growth rate of *Alternaria tenuis* cultures on potato-dextrose agar in Petri plates.

pheres were maintained by constant renewal with mixtures of carbon dioxide and ordinary air combined by means of flowmeters.

Two series of experiments were made in which *Alternaria* was grown on potato-dextrose agar in Petri plates. In each series five plates were used under each condition. In the series reported in figure 11 the carbon dioxide was maintained at approximately 30 percent, and in the series reported in figure 12 at approximately 50 percent. Similar lots of plates were held at the various temperatures in ordinary air. After 2 days' treatment with carbon dioxide the plates were removed from the prepared atmospheres and held with the control plates at the original temperature.

It was found in general that the 2 days' exposure to atmospheres containing 30 percent of carbon dioxide delayed the growth of the fungus about 1¼ days and that a similar exposure to atmospheres containing 50 percent of carbon dioxide caused a delay of approximately 1½ days. The delay was fully as great at the lower as at the

TABLE 2.—*Effect of temperature upon the development of spots on figs that had been inoculated with a spore suspension of Alternaria or Cladosporium*

[Isolations from California figs]

Fungus and lot No.	Period of storage	Holding temperature after storage	Period held	Spots per fruit in lots removed from—				
				32° F.	41° F.	50° F.	59° F.	68° F.
Alternaria:	*Days*	*° F.*	*Days*	*Number*	*Number*	*Number*	*Number*	*Number*
3	4		0	1.7			31.8	
4	3	70	1	.3			3.5	
5	2	70	1	.8			6.8	
6	2	70	1	.1			.7	
8	2	70	1	.1			1.6	
9	2		0	.4	0.8	1.9		
10	2		0	.1	.7	1.6		
11	3	50	2	1.0	.6	2.0		
12	2	50	3	4.4	10.3	13.8		
13	2	50	3	1.8	1.4	3.8		
14	3		0	.0	.0	.3	.3	1.4
15	2	41	5	2.5	1.4	2.0	.9	6.6
16	5		0	.0	.0	.5	1.2	13.1
Cladosporium:								
14	3		0	.0	.1	.9	.7	1.8
15	2	41	5	1.6	2.6	2.9	8.7	12.6
16	5		0	.0	.0	.5	.7	4.7

higher temperatures. It is evident that during the period of treatment with carbon dioxide the activity of the fungus must have been reduced in the first case to roughly one-third of normal and in the second to about one-fourth of normal. At the higher temperatures this inhibition was slightly less than that resulting from a drop of 18° in temperature, as shown in figures 11 and 12.

The results from the carbon dioxide experiments with fruit are shown in table 3. Exposure at 59° or 68° F. to atmospheres containing 35 percent or more of carbon dioxide usually gave as great inhibition of spotting as holding at 32° without carbon dioxide. The results following *Alternaria* inoculations were somewhat more decisive than those following *Cladosporium* inoculations.

The figs of lot 7 (p. 480) were given carbon dioxide treatments similar to those reported in table 3, but the spotting was obscured by the development of *Rhizopus* sp. The carbon dioxide held this fungus in check as well as it held *Alternaria*.

In other experiments with fruit the carbon dioxide treatments were given in pony refrigerators similar to those used in the commercial shipment of Florida strawberries. The carbon dioxide gas was obtained by placing a few pounds of solid carbon dioxide in the refrigerator pan along with ordinary ice. With this method of treatment both the carbon dioxide and the temperature were lower at the end of the experiment than at the beginning. The conditions and the results of the experiments are shown in table 4.

In the six instances in which the carbon dioxide averaged 23 percent or more, the inhibition of spotting was as great as or greater than that resulting from immediate storage at 32° F. The effect was approximately equivalent to that of a drop of 20° in temperature. In the two instances in which the percentage of carbon dioxide was lower than 23 percent, the spotting was worse than that obtained by immediate storage at 32°, but decidedly less than that obtained in the control refrigerators without carbon dioxide.

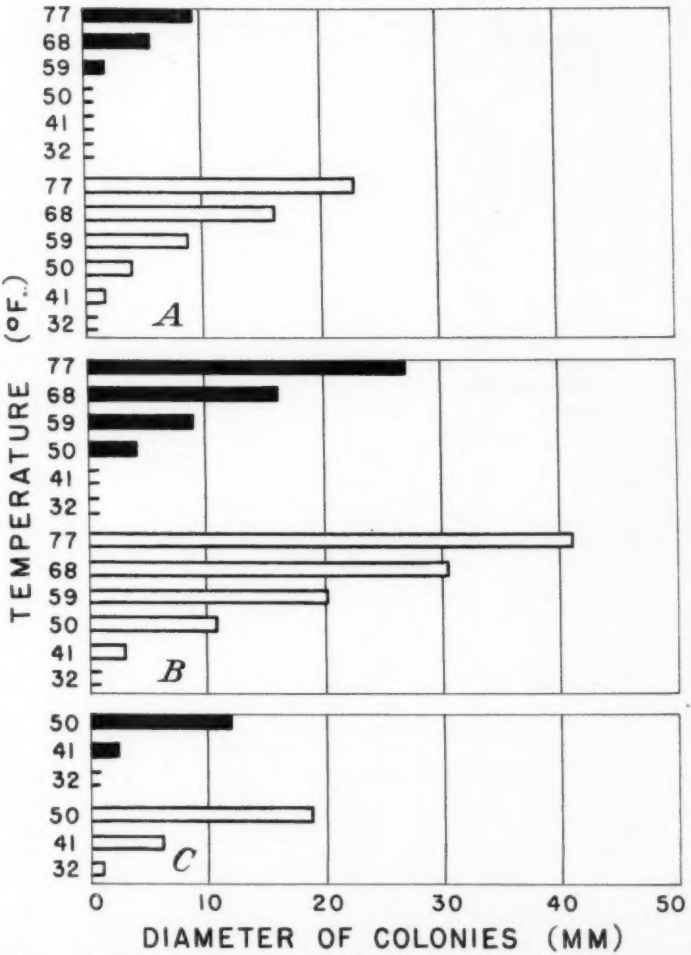

FIGURE 11.—Effect of carbon dioxide at various temperatures on growth rate of cultures of *Alternaria tenuis* on potato-dextrose agar in Petri plates. After 2 days' storage in air containing about 30 percent CO_2, the treated lots were placed with corresponding control lots and continued at the original storage temperatures. Graphs show diameters of colonies at end of (*A*) 48 hours, (*B*) 80 hours, and (*C*) 120 hours. Solid bars, lots treated with CO_2 for first 48 hours; open bars, lots stored without CO_2.

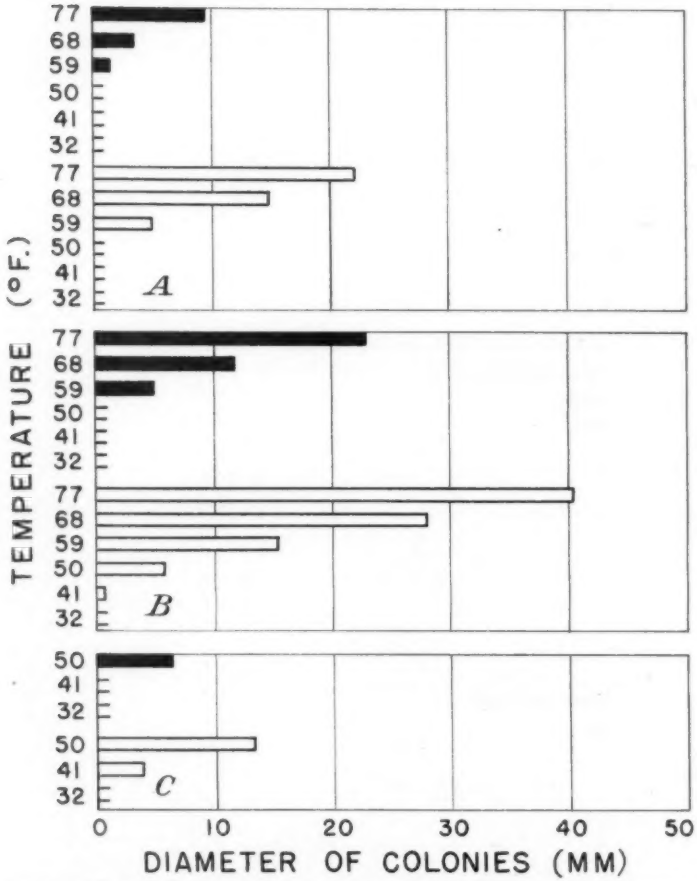

FIGURE 12.—Effect of carbon dioxide at various temperatures on growth rate of cultures of *Alternaria cucumis* on potato-dextrose agar in Petri plates. After 2 days' storage in air containing about 50 percent CO_2, treated lots were placed with corresponding control lots and continued at the original storage temperatures. Graphs show diameters of colonies at end of (*A*) 48 hours, (*B*) 84 hours, and (*C*) 120 hours. Solid bars, lots treated with CO_2 for first 48 hours; open bars, lots stored without CO_2.

TABLE 3.—*Effect of carbon dioxide upon the spotting of figs inoculated with Alternaria or Cladosporium*

Fungus and lot No.	Experimental period	Temperature during experimental period	Carbon dioxide during experimental period	Period held after treatment	Holding temperature after treatment	Spots per fruit
Alternaria:	*Days*	*° F.*	*Percent*	*Days*	*° F.*	*Number*
3	4	59	50	0	----------	0.1
		59	35	0	----------	1.8
		59	0	0	----------	31.8
		32	0	0	----------	1.7
4	3	59	50	1	70	.6
		59	0	1	70	4.2
		32	0	1	70	.3
5	2	59	50	1	70	.8
		59	0	1	70	6.8
		32	0	1	70	.8
6	2	59	50	1	70	.1
		59	0	1	70	.7
		32	0	1	70	.1
8	2	59	50	1	59	.0
		59	0	1	59	1.6
		32	0	1	59	.1
14	3	68	35	0	----------	.0
		68	0	0	----------	1.3
		32	0	0	----------	.0
		41	0	0	----------	.0
		50	0	0	----------	.3
		59	35	1	50	.0
		59	0	1	50	.5
		32	0	1	50	.2
		41	0	1	50	.2
		50	0	1	50	.6
15	2	68	35	5	41	1.5
		68	0	5	41	6.6
		59	35	5	41	.9
		59	0	5	41	4.7
		41	0	5	41	1.4
		50	0	5	41	2.0
Cladosporium:						
14	3	68	35	0	----------	.0
		68	0	0	----------	1.2
		32	0	0	----------	.0
		41	0	0	----------	.1
		50	0	0	----------	.9
		59	35	1	50	.0
		59	0	1	50	2.0
		32	0	1	50	.1
		41	0	1	50	.2
		50	0	1	50	.4
15	2	68	35	5	41	2.1
		68	0	5	41	12.6
		59	35	5	41	2.9
		59	0	5	41	8.7
		32	0	5	41	1.6
		41	0	5	41	2.6
		50	0	5	41	2.9

TABLE 4.—*Effect of carbon dioxide upon the spotting of figs* [1]

Lot No.	Period of treatment	CO_2 in atmosphere during treatment			Temperature during period of treatment			Period held after treatment	Holding temperature after treatment	Spots per fig
		Early high	Final low	Average	Initial high	Final [2]	Average			
	Days	*Percent*	*Percent*	*Percent*	*° F.*	*° F.*	*° F.*	*Days*	*° F.*	*Number*
10	2	55	30	39	69	50	61	0	-------	0.0
10	2	34	14	22	69	53	56	0	-------	.3
10 [3]	2	-------	-------	-------	69	47	51	0	-------	.9
10 [4]	2	-------	-------	-------	32	32	32	0	-------	.1
10 [4]	2	-------	-------	-------	41	41	41	0	-------	.7
11	3	64	25	40	75	49	52	0	-------	.6
11	3	46	3	26	75	49	49	0	-------	.7
11 [3]	3	-------	-------	-------	75	48	47	0	-------	2.5
11 [4]	3	-------	-------	-------	32	32	32	0	-------	1.0
12	2	47	30	38	72	47	50	3	50	3.1
12	2	38	11	23	72	42	46	3	50	3.6
12 [3]	2	-------	-------	-------	72	44	47	3	50	9.1
12 [4]	2	-------	-------	-------	32	32	32	3	50	4.4
12 [4]	2	-------	-------	-------	41	41	41	3	50	10.3
13	2	64	33	44	54	47	46	3	50	1.9
13	2	27	2	12	54	44	44	3	50	3.2
13 [3]	2	-------	-------	-------	54	42	42	3	50	6.4
13 [4]	2	-------	-------	-------	32	32	32	3	50	1.9

[1] The carbon dioxide treatments were given in pony refrigerators. The figs were sprayed with an *Alternaria* spore suspension several hours before the experiments were started.
[2] The final temperature was not always the lowest.
[3] Control; not treated with CO_2.
[4] Placed immediately in low-temperature storage.

The figs were carefully tested as to flavor after the various carbon dioxide treatments. No difference could be detected between the treated and the untreated fruit.

It is evident that in the absence of satisfactory precooling facilities, valuable protection can be obtained by the proper use of solid carbon dioxide.

SUMMARY

The spotting of figs on the market is found to be due mainly to *Alternaria tenuis* Nees, but *Cladosporium herbarum* (Pers.) Link is also capable of producing spots.

Experiments are reported on the effects of humidity, temperature, and carbon dioxide upon the development of spotting.

Spotting was decreased by lowering the humidity of the storage atmosphere, but humidities that gave satisfactory control also caused severe shriveling.

The growth rate of the *Alternaria* on potato-dextrose agar was approximately twice as great at 77° as at 59° F., nearly three times as great at 59° as at 41°, and fully three times as great at 41° as at 32°.

At temperatures of 41°, 50°, 59°, 68°, and 77° F., exposure to 30 percent of carbon dioxide reduced the activity of the *Alternaria* to about one-third of normal.

With temperature conditions somewhat less satisfactory than those usually found during the first 2 days in a nonprecooled car, exposure to atmospheres in which the carbon dioxide averaged 23 percent or more gave as good control of spotting as immediate storage at 32° F.

EFFECT OF LOW CONCENTRATIONS OF COPPER ON GERMINATION AND GROWTH OF CONIDIA OF SCLEROTINIA FRUCTICOLA AND GLOMERELLA CINGULATA [1]

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INTRODUCTION

Spores of a fungus attached to a surface that has been sprayed with a copper-containing fungicide such as bordeaux mixture are in contact with a residue which, when wet, becomes a dynamic physical and chemical system. Although the quantity of soluble copper and the concentration of ions in this system fluctuate with changing conditions, its toxicity to fungus spores appears to be correlated with the available copper originally in the residues or later built up in them, as Goldsworthy and Green (*7*)[2] have shown. It has been demonstrated by Ribéreau-Gayon (*13*), Hockenyos (*9*), McCallan and Wilcoxon (*11*), and Goldsworthy and Green (*7*) that the clear supernatant liquid of freshly prepared bordeaux mixture spray contains several parts of copper per million. McCallan and Wilcoxon (*11*) have shown that, when bordeaux preparations are dried on glass surfaces and rewet, the soluble copper amounts to 0.2 to 0.3 p. p. m.

The data presented by Goldsworthy and Green (*7*) appear to demonstrate that it is the available [3] copper in the dried but rewet bordeaux mixture residues that is responsible for the toxicity of the residues to the conidia of *Sclerotinia fructicola*. McCallan and Wilcoxon (*11*), on the other hand, concluded that the small solubility of the copper in these residues is not sufficient to cause toxicity. They state that "this concentration of copper is insufficient to affect materially the germination of spores of the species of fungi used." This statement may be true of a system in which the concentration of copper is immediately lowered by absorption and not replaced, but it appears logical to assume that in a dynamic system, such as that which is presented by a drop of water in contact with a residue of bordeaux mixture, copper ions absorbed by the spores would be immediately replaced from the residue and the available copper would be sufficient to cause toxic effects. This explanation of such a system is in accordance with the oligodynamic theory of Nägeli (*12*).

The present paper reports the results of experiments on the toxicity of low concentrations of ionized copper, in copper sulphate solutions and in saturated solutions of spray materials containing relatively insoluble copper, to the conidia of *Sclerotinia fructicola* (Wint.) Rehm and *Glomerella cingulata* (Ston.) Spauld. and Schrenk. In conjunction with these experiments, studies were also made on the toxicity of ionized copper when added to various substrates and forming a system considered as static since the original concentration of copper ions was not continuously maintained.

[1] Received for publication August 30, 1937; issued May 1938.

[2] Reference is made by number (italic) to Literature Cited, p. 504.

[3] The "available" copper is defined as that copper which may be readily absorbed by the conidia, with toxic effects, and which may or may not be ionic in nature.

MATERIALS AND METHODS

The conidia used in these experiments were from artificial cultures grown at room temperature and of an age previously determined to be that at which the highest percentage of the conidia were viable. For *Sclerotinia fructicola* growing on potato hard agar and on string beans, this age was 14 days and 7 days, respectively; for *Glomerella cingulata* growing on potato hard agar, it was 4 days. The conidia of *S. fructicola*, after being freed from the surface of the various media either by washing or by suction, were placed in a small amount of distilled water in which they were made to stay in suspension by the addition of a minute quantity of the preparation described by Allison, Hoover, and Burk (*1*) as coenzyme R. The conidia of *G. cingulata* were washed free from their conidiophores with distilled water. The conidia of both species were then seeded in water agar and were distributed so that under the lowest power of the microscope 8 to 12 conidia were in the field at one time. Small blocks of the conidia-containing water agar, 1 by 4 by 4 mm, were then placed in the perfusion apparatus described in the next paragraph, or on agar containing different nutrients and various amounts of copper.

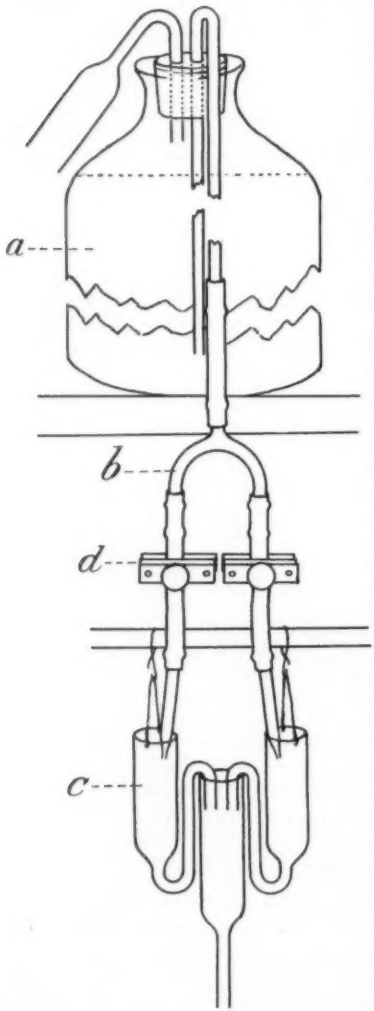

FIGURE 1.—Perfusion apparatus: *a*, Test solution; *b*, siphon system; *c*, perfusion cells; *d*, screw clamps.

The perfusion apparatus (fig. 1) consisted of a supply bottle, containing 2 liters of test solution (*a*), from which the solution was delivered by gravity through a siphon system (*b*) to the perfusion cells (*c*). The flow into the perfusion cells was regulated by screw clamps (*d*), so that the 2 liters of solution in the supply bottle passed over the conidia-containing water-agar cubes during a 24-hour period. The perfusion cells in which the water-agar blocks were placed were built so as to maintain a constant level of liquid by an overflow siphon while allowing a charge of conidia-containing units to be continuously washed with the solution to be tested. In this manner the conidia were subjected to a constant concentration of soluble and

ionized copper, and the amount of copper absorbed could be deduced by determining the total copper remaining in the overflow from the perfusion cells.

The system just described is dynamic and closely simulates the system in operation on a sprayed plant where, under weather conditions favorable to infection, conidia are suspended in a drop of rain on the surface of a film of residue. The drop of rain becomes saturated with the soluble constituents of the residue and remains so until dried up by a change in environmental conditions. If the drop of rain remains long enough the conidium begins activity and then absorbs copper, which is replaced from the residue. The system operates until the conidium is killed or inhibited, provided the store of available copper in the residue is not too small. Such a system is probably not static, because the small amount of copper dissolved in the raindrops on these residues is being continuously replaced after it is absorbed by conidia.

The perfusion apparatus afforded an opportunity to study the reactions of conidia immersed in a saturated solution of a fungicide but separated from contact with the undissolved particles. The rate at which this solution was renewed was such that absorption of copper by the conidia could not significantly change the concentration.

In many of these tests the copper-containing solution was the primary saturated solution of various relatively insoluble copper materials, obtained by shaking intermittently for 48 hours 20 grams of each material with 2 liters of distilled water. The primary solution is defined here as the solution obtained from the first washing; it probably contains, in addition to the soluble portion of the copper material, slight amounts of other soluble ions that were present when the copper material was precipitated out of solution. In addition to these, especially with the commercial compounds used in the experiments, relatively large amounts of various kinds of wetting or stabilizing agents may also be present in the primary solution. After the undissolved residue of these solutions had settled out, which usually required several days, the liquid portion was filtered off by gravity or suction through a Chamberland-Pasteur filter cylinder having an L2 porosity. The filtrate obtained in this manner was then used in the perfusion apparatus. Similar filtrates were tested for their copper content, and in many cases the perfused filtrates were collected and tested for copper.

Since the solutions were clear, no particular caution was necessary in securing samples for analysis. To improve the accuracy of the analysis, it seemed desirable to take a relatively large sample and evaporate it to small volume after adding about 5 ml of nitric acid to make sure that the copper compound present did not come out of solution.

With certain of the organic copper compounds the deep color of the concentrated solution showed plainly that the amount of copper was far beyond the range of the micromethod. These samples were transferred to evaporating dishes, evaporated to dryness, and ignited below red heat in the electric muffle furnace. After cooling, the ash was dissolved in dilute nitric acid and transferred to volumetric flasks, from which portions were taken for analysis by the iodometric method recommended for the determination of copper in fungicides by the Association of Official Agricultural Chemists (*3*).

Haas and Quayle (*8*) have proposed a method for the determination of small quantities of copper that is essentially a small-scale modification of the method mentioned above. The thiosulphate solution is diluted to 0.002 normal from 0.10 normal, but the other steps in the procedure are followed exactly. In this method the cuprous iodide formed remains in solution, yet there seems to be no difficulty in determining the end point. From results with this method it was thought that the determination of intermediate quantities of copper could be made by using intermediate dilutions of the thiosulphate. This was found to be true, and for such quantities of copper this method was employed. The titer of the thiosulphate solution was determined on the day of use by including electrolytically standardized solutions of copper sulphate along with the samples to be analyzed.

The procedures described are scarcely suitable for the determination of less than 0.2 mg (200 micrograms) of copper, because the titer of a 0.002-normal solution of sodium thiosulphate is about 140 micrograms of copper per million. Of the procedures tried, the method of Callan and Henderson (*4*), as modified by Delage (*5*), was found the most convenient for quantities of copper between 10 and 200 micrograms. No attempt was made to determine smaller amounts, because the necessary special precautions, such as the use of purified reagents and purified water, were not taken.

During the period of operation of the perfusion apparatus, the test conidia were examined from time to time. The small cubes of conidia-containing water agar were fished out of the perfusion cell and tested for germination either under the microscope or on nutrient media. Since the perfusion apparatus does not entirely exclude contaminating organisms, resistant contaminants occasionally survived the treatments and grew after transfer to culture media.

In addition to the dynamic system just described, a static system was used which consisted of placing the small cubes of conidia-containing water agar on the surface of water-agar, Czapek's-agar, and potato-agar slants to which dilute solutions of copper sulphate had been added. The copper solutions added to these media were diluted by adsorption by the agar, fixation by the various nutrients, and absorption by the conidia. Copper ions react specifically with alcohol hydroxyl groups, and in every one of the above media, even the plain water agar, almost every constituent has such groups. In these systems, therefore, little was known concerning the active concentration of copper. After a 24-hour period of contact with the copper-containing medium, the conidia-containing blocks were transferred to copper-free nutrient media to test the germinability of the conidia.

EXPERIMENTS WITH THE PERFUSION APPARATUS

EFFECT OF DISTILLED WATER

Distilled water is not usually a good medium in which to germinate the spores of fungi. Why this is true is not clear, since it is agreed by botanists that the spore of a fungus usually contains the means to initiate growth. It would seem logical to assume, provided plasmoptysis does not take place, that the presence of pure water at the proper temperature would be sufficient to cause a swelling of the protoplasm, an activation of the enzymes, and resultant growth. But

this is not always so, as Duggar (*6*) has pointed out, and even among the spores of the same organism there is great variability in the response to distilled water.

Germination in distilled water can be greatly invigorated, however, as Duggar (*6*) and Wilcoxon and McCallan (*15*) have shown, by the addition of some accessory substance, such as a trace of malic acid or some plant decoction. These responses indicate strongly that the spores are dependent on some outside source of energy or stimulation before growth can begin.

Smart (*14*) indicated that a supplementary or external supply of food was necessary to the proper germination of the spores of a number of Myxomycetes. He further showed that single spores do not germinate, but that masses of spores favor germination and that single spores placed in an environment previously inhabited by germinating spores will germinate freely. These facts surely indicate that the spores of species of Myxomycetes are dependent upon some influence other than that furnished by the water for the stimulation of germination. This "autocatalytic" agent probably may be better regarded as some particular nutritional substance derived from the external food supply.

When spores are formed in contact with a food-containing medium, it is possible that all of them, on becoming detached, carry a supply of food or growth-promoting materials on their surfaces. The varying degrees of germination so often observed in distilled water may be due to the varying quantities of these materials carried by the individual conidia. That the conidia of various species have on their surfaces different amounts of materials that stimulate growth is indicated by the work of McCallan and Wilcoxon (*11*). These investigators also show that hydroxy acids are present on conidia derived from nutrient media and that the same or similar materials may be isolated from the surface of used and sterile nutrient agars. While they assume that the hydroxy acids (e. g., malic acid) and other materials isolated from conidia are secreted by conidia, they have not demonstrated that these materials are not also carried over from the medium in which the conidia are grown. Their washing experiments, which show that the assumed secretion may be almost entirely removed in the primary washing of the conidia, appear to indicate that these materials may have been taken up from the medium on which they had grown or that, if secreted by the organism, they are secreted slowly and therefore are not readily replaced. The findings of McCallan and Wilcoxon in general indicate that conidia from a nutrient substrate have adsorbed on their surfaces nutritive materials that may help to initiate germination.

In the perfusion tests described in this paper in which distilled water was the perfusion solution and water agar the suspension medium, germination of the conidia of *Glomerella cingulata* was usually only 6 to 36 percent and that of *Sclerotinia fructicola* rarely more than 5 percent. In all cases the germ tubes were subnormal, both in length and in vigor.

Experiments of various types were conducted to find out why the conidia of both species failed to germinate to their full capacity under these conditions. When the perfused conidia of both species were transferred to water-agar surfaces that had been freed as nearly as possible of all traces of nutrient materials by dialysis, only a slight

increase in germination occurred; but when the transfer was made to a nutrient medium, such as potato agar, germination was more rapid and more vigorous than normal and the percentage of germination was higher. It appears, therefore, that both species are dependent upon certain materials to stimulate germination, and that these materials are removed when the conidia are subjected to a period of washing and are restored when the conidia are transferred to a nutrient medium.

These conidia also appeared to have been activated by the perfusion treatment to a point where the addition of stimulatory substances caused immediate growth. It is not clear why the conidia of *Glomerella cingulata* germinated more freely during the perfusion process than did those of *Sclerotinia fructicola*. That conidia of *S. fructicola* collected by suction are reasonably free of soluble materials from the culture media may account for the lower percentage of initial germination always observed with this species. On the other hand, conidia of *G. cingulata* cannot be collected cleanly from the surface of the culture medium by suction, but generally must be removed by washing. These washed conidia carry over to the water-agar block a varying quantity of soluble nutrient materials that may account for the differences in germination observed between the two species.

In the perfusion tests, the percentage of germination of *Sclerotinia fructicola* conidia collected from string bean culture surfaces by suction was always lower than that of the conidia collected by washing. If the conidia of both *S. fructicola* and *Glomerella cingulata* are suspended in potato or potato-dextrose-agar blocks, which of course contain food materials, instead of water-agar blocks, which contain practically none, and are subjected to perfusion with distilled water, most of the conidia germinate and put out robust germ tubes in a few hours. As the washing continues, with consequent removal of the soluble food and accessory materials, the rate of growth of the germ tube diminishes and its final increment of growth becomes very thin as though the conidium had been starved. When these germinated conidia are placed in contact with a ready supply of soluble nutrients, they begin to grow again and to show the characteristics associated with sufficient nourishment. These experiments indicate that the initial growth in water- and nutrient-agar blocks is made at the expense of some soluble accessory substance which is removed by the perfusion operation.

The perfused conidia appeared not to be injured or changed in any manner by the distilled-water treatment, since it was always found that when perfused conidia were transferred to a suitable medium the percentage of germination was as high as that of unperfused conidia. Although the perfused conidia seem to be arrested in their development by the distilled-water treatment, they appear, as shown by the following experiments, to be able to absorb mineral constituents added to the perfusion liquid.

EFFECT OF LOW CONCENTRATIONS OF IONIZED COPPER

In the determination of the effect of low concentrations of ionized copper on the conidia of *Sclerotinia fructicola* and *Glomerella cingulata* in the perfusion apparatus, copper sulphate in distilled water, at copper concentrations of 4.0, 3.0, 2.0, 1.0, 0.5, and 0.25 p. p. m. was used. A number of experiments were conducted, and the results

of most of them agree with those reported in tables 1 and 2. The slight differences between the data in table 1 and table 2 are accounted for by the variations in the final germinability of the check conidia of *S. fructicola*. The toxicity of the higher concentrations was so quickly apparent that by the time activity was noticeable in the check conidia plasmolysis had occurred in the treated conidia. Since with the lowest dilutions (0.50 and 0.25 p. p. m.) the toxic effects developed more slowly, a more detailed study of the progress of the reaction was made. The data presented in table 2 are characteristic of the manner in which the conidia of both species behave when subjected to very low concentrations of copper ions. The absorption of copper was delayed, possibly by the lack of activity on the part of the organism or possibly by the initial absorption or inactivation of copper by the agar, but as the experiment progressed most of the conidia of both species were killed. In most cases a few conidia survived the treatments, even when the solutions had a high concentration. Apparently these conidia remained dormant or their activity was so slight that they absorbed sublethal quantities of copper.

TABLE 1.—*Toxic effect of low concentrations of copper sulphate solutions on the conidia*[1] *of Sclerotinia fructicola and Glomerella cingulata after a 24-hour perfusion period*[2]

Species	Copper concentration	Condition of conidia at end of perfusion period	Subsequent germination	
			Treated conidia	Check conidia[3]
	P. p. m.		*Percent*	*Percent*
S. fructicola	4.0	Nearly all plasmolyzed	6	98
	3.0	None have germinated; many distinctly plasmolyzed; a few appear turgid and normal.	4	96
	2.0	None have germinated; none appear turgid; all plasmolyzed.	8	98
	.50	None have germinated; all appear plasmolyzed	6	96
	.25	Some appear not injured; many appear plasmolyzed; a few have germinated.	16	94
G. cingulata	4.0	All appear plasmolyzed	0	100
	3.0	do	0	98
	2.0	do	0	100
	1.0	Nearly all cells appear plasmolyzed; an occasional cell appears turgid.	[4] 2	100
	.50	About 8 percent have germinated, but all appear plasmolyzed.	[4] 4	98
	.25	About 10 percent have germinated; most appear plasmolyzed; a few appear turgid.	[4] 2	100

[1] *S. fructicola* conidia were collected from 7-day-old cultures growing on string bean suspended in distilled water plus a small amount of coenzyme R; *G. cingulata* conidia were collected from 4-day-old potato-agar cultures by being washed off with distilled water.
[2] Period allowed for passage of solution over conidia suspended in water-agar blocks.
[3] Check conidia, also suspended in water-agar blocks, were perfused with distilled water.
[4] Continued activity.

The results obtained show that both species are very sensitive to low but constant concentrations of copper ions, once growth activity has begun and an equilibrium has been reached between the copper solution and the agar. Since spraying tests in the field indicate that *Glomerella cingulata* is more resistant to copper than *Sclerotinia fructicola*, it is generally supposed that the conidia of *G. cingulata* are more resistant to copper. Under the conditions of these experiments, when the conidia are deprived of their adherent supplements, it may be concluded that both species are equally susceptible to ionized copper. Under natural conditions, conidia of *G. cingulata* are usually

covered with a mucilaginous material that may bring about apparent resistance to copper sprays by the fixing or inactivation of ionized copper.

TABLE 2.—*Progressive toxic effect of low concentrations of copper sulphate solutions on the conidia of Sclerotinia fructicola and Glomerella cingulata* [1]

Original concentration of copper	Concentration of copper at end of perfusion period	Perfusion period [2]	Subsequent germination of—			
			Treated conidia		Check conidia [3]	
			S. fructicola	*G. cingulata*	*S. fructicola*	*G. cingulata*
P. p. m.	*P. p. m.*	*Hours*	*Percent*	*Percent*	*Percent*	*Percent*
0.25	----------	1.0	82	98	88	98
.25	----------	2.0	56	78	88	98
.25	----------	4.5	48	66	84	98
.25	0.21	24.0	16	8	86	98
.50	----------	1.0	74	98	88	98
.50	----------	2.0	48	82	88	98
.50	----------	4.5	33	46	84	98
.50	.48	24.0	14	6	86	98

[1] *S. fructicola* conidia were collected from 7-day-old cultures growing on sterile bean; *G. cingulata* conidia were collected from 7-day-old potato-agar cultures.
[2] Period allowed for passage of solutions over the conidia suspended in water-agar blocks.
[3] Check conidia, also suspended in water-agar blocks, were perfused with distilled water for 24 hours.

In a few experiments with copper concentrations of 0.25 and 0.50 p. p. m., the perfused filtrates were collected and analyzed for residual copper. It was usually found that only a small proportion of the original copper was absorbed by the conidia (table 2, column 2). Staining the killed conidia with the copper-sensitive chromotropic acid (*2*) indicator demonstrated that a quantity of copper sufficient to furnish a distinct color reaction had been absorbed and fixed by them. Since in another part of this paper the authors show that agar also has the property of absorbing copper, it appears that the loss of copper during perfusion is accounted for by absorption on the part of both conidia and agar. These experiments indicate that only a very small amount of ionic copper was necessary to inactivate a great number of conidia, and it seems logical to believe that there is sufficient available copper associated with bordeaux mixture residues to be toxic.

EFFECT OF SATURATED SOLUTIONS OF VARIOUS COPPER COMPOUNDS

Saturated solutions of various copper compounds were obtained by shaking intermittently by hand, at room temperature, over a period of 48 hours, 20 g of the copper materials with 2 liters of distilled water. After the undissolved material had settled out, the supernatant liquid was filtered through an L2 Chamberland-Pasteur filter candle either by gravity or by suction. The clear primary solution thus obtained was then employed in the perfusion tests and also in the analytical determinations. However, it was found that the perfusion tests could be conducted with the supernatant fluid without filtering, since the agar of the water-agar blocks apparently prevents the small amount of the solid phase which is unavoidably present from coming in contact with the suspended conidia. The perfusion tests were conducted in the same manner as those described previously. The results are shown in table 3.

TABLE 3.—*Toxic effect of primary saturated solutions obtained from various copper compounds on the conidia of Sclerotinia fructicola and Glomerella cingulata* [1]

Copper compound	Subsequent growth of test conidia after being treated with primary perfusion solution for—								Solubility of primary saturated solution
	2 hours		4 hours		6 hours		24 hours		
	S. fructicola	*G. cingulata*	*S. fructicola*	*G. cingulata*	*S. fructicola*	*G. cingulata*	*S. fructicola*	*G. cingulata*	
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*P.p.m.*
Phosphate	100	100	100	100	100	100	98	100	0.17
Silicate	100	100	96	98	96	100	92	100	.48
Ammonium silicate	100	100	100	100	100	100	100	100	.20
Oxide (cupric)	100	100	100	100	98	100	100	100	.11
Oxide (cuprous)	45	42	12	Trace	4	0	0	0	26.7
Oxychloride:									
1	15	18	12	8	8	4	0	0	160.0
2	100	100	92	98	78	100	8	100	8.0
Basic sulphate	91	86	82	72	46	21	0	1	11.5
Zeolite:									
1	100	100	100	100	100	100	98	100	.05
2	100	100	100	100	100	100	96	100	.23
Malate	100	82	100	78	100	81	68	12	1,240.0
Maleate	86	0	46	0	21	0	Trace	0	315.0
Basic maleate	92	0	93	0	25	0	Trace	0	494.0
Resinate	100	100	97	100	96	100	100	100	.086
Oxalate	100	100	100	100	100	100	31	100	4.3
Check [2]							100	100	

[1] *S. fructicola* conidia collected from 7-day-old bean culture; *G. cingulata* conidia, from 4-day-old potato-agar culture.
[2] Check conidia were perfused with distilled water for 24 hours.

The copper compounds tested were, for the most part, commercial materials that showed some promise of becoming useful as fungicides. Of the inorganic materials, black copper oxide, copper silicate, copper ammonium silicate, copper phosphate, and copper zeolites were found to be nearly insoluble, and the copper in solution appeared to be either in an unavailable combination or available in quantities so minute that toxicity was negligible. Most of these materials, when tested in field experiments against the apple scab organism (*Venturia inaequalis* (Cke.) Winter) have not proved sufficiently useful to replace lime-sulphur solution during the seasons in which the disease is severe. They are useful, however, when mild fungicides that are relatively noninjurious to apple trees may be safely used.

The primary saturated solutions of red copper oxide, basic copper sulphate, and one of the copper oxychlorides (1) were consistently toxic to the conidia of both *Sclerotinia fructicola* and *Glomerella cingulata*, and the degree of toxicity appeared to be directly correlated with the amount of soluble copper present. Another one of the copper oxychlorides (2), despite its relatively high solubility, was found to be only slightly toxic to the conidia of *S. fructicola* and nontoxic to the conidia of *G. cingulata*. Why this particular copper oxychloride should show such a low degree of toxicity is not known. The copper solubilities of the two copper oxychloride materials are different, but, according to the authors' findings with ionic copper, the quantity of soluble copper should be sufficient in both cases to be toxic to the conidia of both fungi.

Orchard tests in various parts of the country[4] indicate that the black copper oxide, copper phosphate, copper ammonium silicate, copper zeolites, and copper silicate are not so effective against certain parasitic fungi as the more soluble red copper oxide, basic copper sul-

[4] Unpublished data.

phates, and certain copper oxychlorides. It is certain that red copper oxide, basic copper sulphate, copper ammonium silicate, and copper oxychlorides when used with lime are at times very injurious to the fruit and leaves of certain species of higher plants, whereas black copper oxide, copper phosphate, copper zeolites, and copper silicate are not.

Of the organic materials used, copper resinate, copper oxalate, and copper malate proved to be the least effective, whereas copper maleate and basic copper maleate were very toxic. It appears possible that in these compounds the copper is principally combined in complex molecules, since its solubility varies a great deal among the compounds and since their toxicity is not correlated with their degree of solubility. The solubility of the malate is of a high order, but a saturated solution of this compound is not quite so toxic as a dilute solution of copper sulphate containing a copper concentration of 0.25 p. p. m. The organic copper compounds also bring into solution large quantities of acid radicals that may affect toxicity. The toxic effects of the malate are not constant, possibly because of the enormous amount of malic acid combined with the soluble copper. In the case of copper oxalate, the effect of the acid appears to be even more marked. A saturated solution of copper oxalate was apparently nontoxic to conidia of *Glomerella cingulata* and was only slightly injurious to conidia of *Sclerotinia fructicola*. Nine parts per million of oxalic acid, which is the equivalent of that present in the copper oxalate solution used, appears to exert the same differential effect on the conidia of the two species of fungi. A saturated solution of copper resinate had no noticeable effect on either species. In considering these results, it should be kept in mind that a copper sulphate solution containing copper in a concentration of 0.25 p. p. m. was toxic to the conidia of both species.

The lack of pronounced toxic effect observed in the case of the copper malate solution is of special interest, since it has been pointed out by McCallan and Wilcoxon (*11*) that the conidia of *Sclerotinia fructicola* and *Glomerella cingulata*, organisms used in their experiments, apparently have the power of secreting malic and other hydroxy acids. It was their thought that when these conidia encountered bordeaux mixture residues the hydroxy acids that they secreted dissolved copper. When ionized copper comes in contact with malic acid ions, apparently a very soluble copper malate is formed, but experiments made by the present authors show that the copper is in a nonavailable form and is not particularly toxic to the conidia of the two species. The results of additional experiments dealing with combinations of copper and malic acid ions are presented in another portion of this report. The marked toxicity of normal copper maleate and basic copper maleate indicates that the copper is more available in these compounds and is readily absorbed by the conidia. Maleic acid, while extremely injurious to higher plants, appears not to be deleterious to the conidia tested. By the chromotropic test (*7*), conidia killed by these solutions were shown to have absorbed copper, indicating that the materials are either readily absorbed or that ionization of copper occurs.

The amount of copper present in the primary saturated solutions of most of these compounds is greater than in the lowest concentration of copper in the copper sulphate solutions used in the

previous experiments, yet these experiments reveal that only with certain of these materials was the toxic effect as pronounced. A possible explanation of the failure of copper silicate, a copper zeolite (2), one of the copper oxychlorides (2), and copper oxalate to show the same toxicity as a corresponding concentration of copper sulphate is that the soluble fractions contain complex ions or inactivating compounds which cause the copper to be in an unavailable form.

EXPERIMENTS WITH COPPER SULPHATE ADDED TO CULTURE MEDIA

In these experiments ionized copper, in the form of a sulphate solution, was added to water agar, Czapek's agar, and potato agar. After a considerable number of tests, it was found that the equilibrium point, i. e., the point at which enough ionized copper was added to cause a definite lethal effect, differed according to the medium used and the organism tested. The equilibrium point does not necessarily indicate that all the conidia were killed at that point. It is the point at which lethal effects are first noted and at which germination of most of the conidia is inhibited but may proceed after transfer to culture media. This is shown at the copper concentration of 185 p. p. m. (table 4) where water agar was used as the base or carrier of the copper. It will be observed that at the lower concentrations many of the conidia of both *Sclerotinia fructicola* and *Glomerella cingulata* germinated, usually with germ tubes shorter than those of the check.

TABLE 4.—*Growth responses of conidia of Sclerotinia fructicola and Glomerella cingulata when seeded for 24 hours on plain water agar to which various concentrations of copper sulphate had been added*

Concentration of copper	*Sclerotinia fructicola* [1]			*Glomerella cingulata* [2]		
	Germination	Observations	Subsequent growth [3]	Germination	Observations	Subsequent growth
P. p. m.	*Percent*		*Percent*	*Percent*		*Percent*
62	92	Long germ tubes	96	96	Medium germ tubes	100
125	66	Medium germ tubes	82	62	do	86
185	82	Short to medium tubes; all plasmolyzed.	12	76	do	87
250	12	Short germ tubes	0	56	Short germ tubes	76
312	14	do	0	68	do	78
374	10	do	0	74	do	56
434	9	do	0	72	Short to medium germ tubes	64
500	12	do	0	58	do	62
562	10	do	0	48	do	68
625	12	do	0	52	Some cells plasmolyzed; short germ tubes.	38
Check	96	Long germ tubes	96	100	Medium to long germ tubes	100

[1] Cultures obtained from 7-day-old string bean growths.
[2] Cultures obtained from 4-day-old potato hard-agar growths.
[3] Subsequent growth determined by transferring the treated cubes of conidia-containing water agar to potato hard agar.

At concentrations nearly up to the equilibrium point, the number of conidia that germinated was much less. Especially was this true of the conidia of *Sclerotinia fructicola*, none of which were found active after being subjected for 24 hours to a concentration nearly up to the equilibrium point. A higher percentage of the conidia of *Glomerella cingulata* apparently survived contact with the copper-containing

medium at the equilibrium point, and though inhibited in their growth they continued activity when transferred to culture media. Because the percentage of subsequent survival was greatest where initial growth was most active, it appears that initial growth, by either using up or inactivating some of the copper of the solution, had lowered the copper concentration to a point where the remaining conidia were merely inhibited or not injured at all. Of course, this is the weakness of a static system. One never knows with exactness what the real concentration of any of the components of such a system may be after absorption by conidia has begun. It appears from these tests that conidia of *G. cingulata* were more active absorbers of the toxic materials than were those of *S. fructicola*.

Where *Sclerotinia fructicola* was used as the test organism, the equilibrium point for water agar was between 125 and 180 p. p. m.; for Czapek's agar, 120 to 150 p. p. m.; and for potato agar, 500 to 572 p. p. m. With *Glomerella cingulata* as the test organism, the equilibrium point for water agar was 185 to 250 p. p. m.; for Czapek's agar, 150 to 180 p. p. m.; and for potato agar, 350 to 375 p. p. m. In these static systems the initial concentration of ionized copper necessary to furnish a toxic quantity was vastly greater than that required in the dynamic system. The closeness of the equilibrium points for water agar and Czapek's agar indicates that the sugars and phosphates of the latter do not inactivate much of the copper. Potato agar was found to inactivate a high percentage of ionized copper. Water agar, though free of nutrients, apparently inactivated copper either by simple adsorption or by combination with the alcohol hydroxyl groups of the agar molecules. The complicated and changeable systems presented by the potato agar and synthetic Czapek's agar prevented their use in collecting data suitable for the determination of the equilibrium point. For this reason the water-agar systems were used to obtain the data presented in table 4. It is probable that, as was noticed when a dynamic system was used, the conidia of *Glomerella cingulata*, when transferred from nutrient media to the water-agar blocks, carried over larger quantities of inactivating substances than did the conidia of *Sclerotinia fructicola*. It appears from these experiments that it is futile to carry out tests dealing with the toxicity of metallic ions to conidia in systems where colloids and other materials have to be added to promote activity of the conidia or where activity of the conidia causes chemical changes in the substrate.

The results obtained with the copper-water-agar system appeared to indicate that for *Sclerotinia fructicola* (but not for *Glomerella cingulata*) one could fix with some degree of certainty the range of copper concentrations at which lethal and inhibitory effects on the conidia could be registered. Because experiments with the copper malate equilibrium solutions had indicated that copper ions are rendered unavailable when combined with malic acid ions, it was thought that the addition of an equal amount of malic acid to a system containing a concentration of copper ions known to be lethal to *S. fructicola* would tend to inactivate the soluble copper. Table 5 shows the results of an experiment designed to test this hypothesis. It will be noted that the addition of nearly the same amount of malic acid completely inactivated the copper at concentrations known to be toxic or inhibitory (at or near the equilibrium point). An excess of

malic acid appeared to accelerate the growth of the germ tubes, while lesser amounts allowed some of the copper to become effective. This was especially indicated when comparison was made of the effect of adding malic acid in the proportion of 50 p. p. m. to the minimum and to the maximum equilibrium concentrations respectively. Transfers to nutrient media indicated that 76 percent of the conidia survived the treatment when the solution contained 50 p. p. m. of malic acid and 125 p. p. m. of copper, whereas only 16 percent of the conidia survived when the solution contained the same amount of acid and 180 p. p. m. of copper. It appears from these studies that malic acid inactivates copper and that the copper malate molecule formed is not absorbed by the conidia of *S. fructicola*.

TABLE 5.—*Growth responses of conidia of Sclerotinia fructicola when seeded for 24 hours on plain water agar to which various concentrations of copper sulphate and malic acid had been added*

Concentration		Observations on conidia after 24 hours	Subsequent growth	Observations [1]
Copper	Malic acid			
P.p.m.	*P.p.m.*		*Percent*	
125	50	Many plasmolyzed; 26 percent with short germ tubes	76	Medium to long tubes.
125	100	A few plasmolyzed; 76 percent with medium to short germ tubes.	96	Germinated.
125	150	A few plasmolyzed; 86 percent with short germ tubes	96	Growing; tubes branched.
125	200	94 percent with medium to long germ tubes	96	Heavy growth.
125	250	96 percent with long germ tubes		Do.
125	300	96 percent with long to medium germ tubes	98	Do.
180	50	Mostly plasmolyzed; 15 percent with short germ tubes	16	Medium growth.
180	100	Many plasmolyzed; 18 percent show short germ tubes	76	Long germ tubes.
180	150	Only a few plasmolyzed; 66 percent show short germ tubes.	98	Do.
180	200	None plasmolyzed; 56 percent with short germ tubes	96	Do.
180	250	None plasmolyzed; 92 percent with medium to long germ tubes.	96	Branched tubes.
180	300	None plasmolyzed; 94 percent with medium and long germ tubes.		Do.
0	50	98 percent with long germ tubes	98	Do.
0	150	do	98	Heavy growth.
0	300	92 percent with medium germ tubes		Do.
125	0	A few plasmolyzed; 12 percent show buds or short germ tubes.	18	Medium tubes.
180	0	None germinated; mostly plasmolyzed	11	Short tubes.

[1] Observations were made 24 hours after material was transferred to potato hard agar.

DISCUSSION

These experiments have demonstrated that a very minute quantity of ionized or available copper, if kept constant by continuous replenishment, is toxic to the conidia of *Sclerotinia fructicola* and *Glomerella cingulata*, whereas without replenishment a large quantity of copper in the primary solution is necessary to bring about the same effect. Although it has not been demonstrated that the physicochemical system operating under natural conditions, when a conidium is in contact with a spray residue in a drop of rain, is exactly comparable to either of the systems that have been designated "dynamic" and "static," it seems probable that such a system is more nearly dynamic than static. Until the amount of copper coming into solution becomes less than that taken out, the toxic action of the available copper must be rapid; but it slows down as the ratio of production to absorption becomes less and less. Thus, if a conidium comes in contact with a

fresh bordeaux mixture residue the toxic action is relatively rapid; if the contact is made with a weathered and depreciated residue the action is relatively slow. In the latter case the final effect may be only inhibitory. That such changes in toxic effects actually occur has been demonstrated by Goldsworthy and Green (*7*), who showed that fresh residues of bordeaux mixture are toxic to conidia of *S. fructicola*, whereas weathered residues show varying degrees of toxicity and inhibition.

The results of experiments reported here indicate that organic compounds secreted by or adsorbed on the conidia may have the property of inactivating ionized copper in spray residues. McCallan and Wilcoxon (*11*) have demonstrated that millions of conidia may furnish traces of these materials; they have also indicated (indirectly, of course) that a single conidium must possess a very minute quantity of these materials. The results of the experiments reported here demonstrate that the materials shown by McCallan and Wilcoxon to be secreted by conidia and to have the supposed property of bringing copper into solution also have the property of changing ionized copper to inactive copper. It has been demonstrated also that the conidia of *Sclerotinia fructicola* and *Glomerella cingulata* actually survive in contact with enormous quantities of the copper compounds thus formed.

The static system used shows very definitely that organic materials such as are usually found in ordinary nutrient media are capable of inactivating considerable quantities of soluble copper. That fixation of ionic copper may also be a function of adsorption or combination with alcohol hydroxyl groups is plainly indicated by the inactivation of copper by plain water agar, where no measurable nutrient or inorganic material is present. These results indicate that a conidium in contact with a source of ionic copper may reduce the quantity of copper either by adsorption or by supplying hydroxy acids secreted by it or adsorbed on its surface.

The results obtained from the primary saturated solutions of various inorganic and organic copper compounds definitely substantiate the findings of Holland, Dunbar, and Gilligan (*10*) and Ribéreau-Gayon (*13*) in showing that the degree of solubility does not necessarily indicate the degree of toxicity. It is the available copper, i. e., copper which can be absorbed by the conidia, that is important in this respect. Thus basic copper sulphate, with a solubility of 11.5 p. p. m., was found to be more toxic than a saturated solution of copper malate, with a solubility of 1,240 p. p. m., because the available copper in basic sulphate solution is apparently greater than in copper malate solution; but it was also evident that not all the copper in the saturated solution of the basic copper sulphate exerted a lethal effect, because its toxicity was less than that of a solution of copper sulphate containing copper in a concentration of 4.0 p. p. m. This is probably true of all the saturated solutions tested, based on comparisons with the dilute, fully ionized copper sulphate solutions. Apparently the saturated solutions used in the experiments reported here are similar to those obtained by Ribéreau-Gayon (*13*) from bordeaux mixture in that only a small portion of the copper was actually ionized, or available. From the results obtained with the black copper oxide, copper zeolites, copper phosphate, copper silicate, and copper oxychlorides, it appears that none of the determined solubilities indicate the true availability of the

copper in these solutions. Although the solubilities of the two copper oxychlorides tested are greatly in excess of that shown to be toxic for the completely ionized copper sulphate, only one of them was toxic to a noticeable degree. While the red copper oxide and basic copper sulphate exert a toxic effect, it is not certain that availability and solubility are of the same order.

Although the amount of available rather than soluble copper indicates whether these compounds will be toxic when placed in contact with fungus conidia, their solubilities appear to be positively correlated with injury when in contact with the tender parts of sprayed plants. It has been found that black copper oxide, copper phosphate, copper silicate, and copper zeolites cause little, if any, injury to the leaves of sprayed plants, whereas copper oxychlorides, basic copper sulphate, red copper oxide, copper malate, copper maleate, basic copper malate, and copper oxalate are unsafe in varying degrees, depending on their solubilities. This is especially true of the malate, maleate, and basic maleate, which are the most soluble.

It therefore appears that the injurious effects of a copper-containing compound on sprayed plants may be determined by its degree of solubility and the fungicidal activities by the degree of ionization in its solution. Since most of the materials that are more or less injurious to higher plants appear to have solubilities of more than several parts per million, which is about that of alkaline bordeaux mixtures, it seems safe to predict that but few of these will be less injurious than bordeaux mixture. The ideal copper spray would apparently be one in which the concentration of dissolved copper would be not much greater than the concentration of ionized copper known to be injurious to higher plants. In such a spray the concentration of ionized copper should be high enough to be toxic to fungus spores but not high enough to cause serious injury to the sprayed plant. This concentration appears to be about 1.0 p. p. m.

SUMMARY AND CONCLUSIONS

A study is reported of the effect of low concentrations of copper ions, administered in two different ways or systems, on the activity of the conidia of *Sclerotinia fructicola* (Wint.) Rehm and *Glomerella cingulata* (Ston.) Spauld. and Schrenk.

The system referred to as "dynamic" consisted of furnishing for 24 hours a continuous supply of certain concentrations of copper ions by means of a gravity flow controlled in such a manner as to deliver the solutions to the test conidia embedded in water agar. In this system the copper concentration was kept practically constant.

The system referred to as "static" consisted of exposing the test conidia embedded in water agar to certain concentrations of copper ions added to water agar, Czapek's agar, and potato agar. In this system the copper concentration gradually decreased and was not restored.

The conidia of both species were definitely injured by solutions of copper sulphate having concentrations of ionic copper as low as 0.25 p. p. m. administered in a dynamic system.

The degree of toxicity of ionic copper is higher in a dynamic system, where the factors that favor inactivation or fixation are overcome, than in a static system, where no record of the actual concentration of ionic copper is available once the system is in operation.

The degree of toxicity of copper sulphate solution in the static system varied according to the power of the various media to fix, and consequently to inactivate, copper ions. The amount of fixation was determined by the character and composition of the media and appeared also to be greatly influenced by direct adsorption or by combination with alcohol hydroxyl groups.

Primary saturated solutions of relatively insoluble copper compounds, obtained from the first washing of 20 g of material by 2 liters of distilled water, showed differences in toxicity that apparently were correlated with the amount of available copper and not with the amount of soluble copper in the solutions.

The primary saturated solutions of cupric oxide (black), copper phosphate, copper zeolite, copper ammonium silicate, and copper silicate were nontoxic to the conidia of *Sclerotinia fructicola*, and *Glomerella cingulata;* and one of two copper oxychlorides was only slightly toxic to the conidia of *S. fructicola* and nontoxic to the conidia of *G. cingulata*.

The primary saturated solutions of cuprous oxide (red), basic copper sulphate, one of the copper oxychlorides, copper maleate, and basic copper maleate were toxic to both species.

The primary saturated solution of copper malate, having a copper solubility of 1,240 p. p. m., proved only slightly less toxic than a copper sulphate solution containing a copper concentration of 0.25 p. p. m.

The primary saturated solution of copper oxalate proved to be only slightly toxic to the conidia of *Sclerotinia fructicola* and innocuous to the conidia of *Glomerella cingulata*. Experiments with dilute concentrations of oxalic acid showed that this difference was due to the specificity of the acid.

Ionic copper in quantities sufficient to be toxic to the conidia of *Sclerotinia fructicola* could be entirely inactivated by the addition of an equivalent amount of malic acid. The acid, in low concentrations at least, was assimilated by the conidia of both species, and promoted growth.

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THE ORIGIN OF LINT AND FUZZ HAIRS OF COTTON[1]

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INTRODUCTION

The mature seeds of most varieties of upland cotton are covered with two different types of hairs, (1) the long lint hairs that are extensively used in the manufacture of fabrics, and (2) the short, thick-walled, fuzz hairs (linters) that form a dense, tangled mat close to the surface of the seed. Both lint and fuzz hairs are single-celled, tubular outgrowths that arise from the epidermal cells of the seed coat. Although the principal difference between the lint and fuzz hairs is the much greater length of the lint, it is usually difficult or impossible to draw a sharp line of demarcation between them on this basis. Very short lint hairs and long fuzz hairs may be indistinguishable. It is sometimes possible, however, to distinguish between lint and fuzz by their position on the ovules and seeds. In some varieties of cotton both the lint and fuzz hairs are restricted to specific and sometimes separate areas on the surface of the seed, giving rise to various "patterns." In the so-called naked seed varieties the fuzz hairs are entirely missing, so that removal of the lint leaves the seed coat smooth and free from hairs. In some varieties the lint and fuzz hairs are pigmented differently, and frequently pigment is present in the fuzz when it is entirely absent from the lint hairs. Another indication of an essential difference between these two kinds of hairs is the fact that the pattern of the fuzz-covered areas on the seed may be inherited independently of the lint pattern.

These facts suggest that, although lint and fuzz hairs both arise from the same layer of epidermal cells, some fundamental differences may exist between them. These differences may be the result of variations in the rate of growth of cells that originate at the same time, or lint and fuzz hairs may owe their characteristics to differences in the time or method of their origin. Although the origin and development of lint hairs has been studied, very little is known about the early history of the fuzz hairs, and the early development of the two types appears never to have been studied carefully from a comparative point of view. It is the purpose of this paper to present the results of such a comparative study of the origin and early history of lint and fuzz hairs.

PREVIOUS WORK

Cytological studies of the origin of lint hairs have been undertaken by a number of investigators. Balls (*3*)[2] states that the lint hairs all originate as single-celled outgrowths of epidermal cells which "sprout

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[2] Reference is made by number (italic) to Literature Cited, p. 520.

at the same stage of boll development, on or near the day when the flower opens." According to Balls (*2*), the fuzz hairs, like those of the lint, arise "in much the same way, at the same time, and from the same layer of cells." Singh (*8*) observed new fibers originating for a period of 48 hours after flowering, but Gulati (*6*) presented evidence that fibers continue to form from epidermal cells for as long as 10 days after the opening of the flower. Farr (*4*, *5*), in a cytological study based upon American upland cotton, reported that cotton hairs develop from epidermal cells for a period of 10 or 12 days after the date of flowering.

The period of fiber origin has also been studied in a very different way. Turner (*9*) reported the results of statistical studies of the number of cotton hairs upon ovules of different ages. He found a rapid increase in the number of hairs as the ovules grew older and larger. In one study, 10-day-old ovules were found to have 10,050 hairs, while 1-day-old ovules possessed only 4,530. Turner pointed out that this increase in hair number must mean that cotton hairs continue to arise after the day of flowering. Gulati (*6*) reported results indicating an even greater increase in number of hairs during the early stages of ovule growth. No distinction between lint and fuzz hairs was made in either of these studies.

In studying the variability in length of cotton fibers by statistical methods, Armstrong and Bennett (*1*) came to the conclusion that the number of fibers on the ovules increased until 25 days after the date upon which the flower opened.

Although all investigators agree that cotton hairs arise from epidermal cells on and soon after the day on which the flower opens, there is much disagreement as to the number of days after flowering during which hairs continue to be produced. In none of the studies of fiber origin has any systematic attempt been made to distinguish between lint and fuzz hairs. This study was undertaken in an effort to clarify these points and to determine as accurately as possible the early developmental history of both lint and fuzz hairs.

MATERIALS AND METHODS

Lint and fuzz hairs both arise from the same layer of epidermal cells, and they are frequently indistinguishable in appearance during the early stages of their growth. It is not always possible, therefore, to determine by observation alone whether the outward extension of any given epidermal cell is destined to become a lint hair or a fuzz hair. The fact that certain varieties of cotton have lint hairs but no fuzz and that other varieties produce lint and fuzz hairs in separate and restricted areas on the ovule surface suggested a means of identifying the two types of hairs. This study was made, therefore, by comparing the developmental histories of hairs produced upon naked (i. e., fuzz-free) seeds and pattern types (those in which lint and fuzz hairs are restricted to separate or specific areas on the seed) with seeds that produced both lint and fuzz hairs over their entire surface.

Six varieties of cotton were used in this study. Four of these—Mexican 128–6, King Naked, Cleveland Fuzzy Tip, and Nankeen Lint—are varieties of *Gossypium hirsutum* L., and two—S × P 30

(Sakel × Pima) and a Bleak Hall strain of sea island—are varieties of *G. barbadense* L.

In order to obtain a complete picture of the development of hairs in these varieties, series of ovules were collected in such a way as to give the stages of growth at 24-hour intervals. In the case of Mexican 128–6, three sets of collections were made. On July 11, 217 flowers were tagged, and ovules were collected daily thereafter for 20 days. On July 15 approximately 500 flowers were tagged, and on August 15 1,000 flowers were tagged. Daily collection from these two taggings continued for 45 days. By this method of tagging, the collection from each of the three dates of flowering constituted a reliable growth series which was representative of a particular day of flowering, and the material of successive days had experienced the same environmental conditions during the earlier periods of growth.

Because only a limited amount of material of the other varieties was available, it was necessary to tag all flowers as they opened each day and to collect the entire crop of bolls of a single variety on a day which would afford an unbroken series. In this way, Sakel × Pima material was collected on August 31, sea island on September 2, King Naked on September 15, Nankeen Lint on September 16, and Cleveland Fuzzy Tip on September 24. All collections were made in the afternoons during the summer of 1936.

After collection, the bolls were opened and the young ovules were dropped immediately into fixing agents. A number of different fixing agents were employed, but the most satisfactory results were obtained with the following formulas:

		Cc
(1)	Formalin	4
	Formic acid	4
	50-percent ethyl alcohol	92
(2)	Formalin	4
	Formic acid	4
	N-butyl alcohol saturated with water	92

After fixing, the ovules were dehydrated with n-butyl alcohol. Five- and ten-micron longitudinal sections were cut from 60° paraffin and were stained with Heidenhain's haematoxylin for maximum contrast.

RESULTS WITH VARIETIES STUDIED

KING NAKED

The King Naked variety presents the problem in its simplest form, since lint hairs alone are produced. When the lint hairs are removed from the seed, the seed coats are smooth and entirely free from fuzz (fig. 1, *A*). The mature seeds bear lint hairs over the greater portion of their surface, only the lower fifth or sixth of the seed coats being entirely devoid of fibers (fig. 1, *H*).

The simplicity of this fiber pattern is reflected at an early age by a comparable simplicity in the time and place of origin of fibers on the growing seed. On the day of flowering, the epidermal layer of the ovule gives no evidence of young fibers or fiberlike processes. Twenty-four hours later, the first fiber initials are seen developing on the chalazal half of the ovule. On the second day after flowering, these fiber initials have elongated noticeably, and no new outgrowths are ordinarily seen on this part of the ovule during this or at any subse-

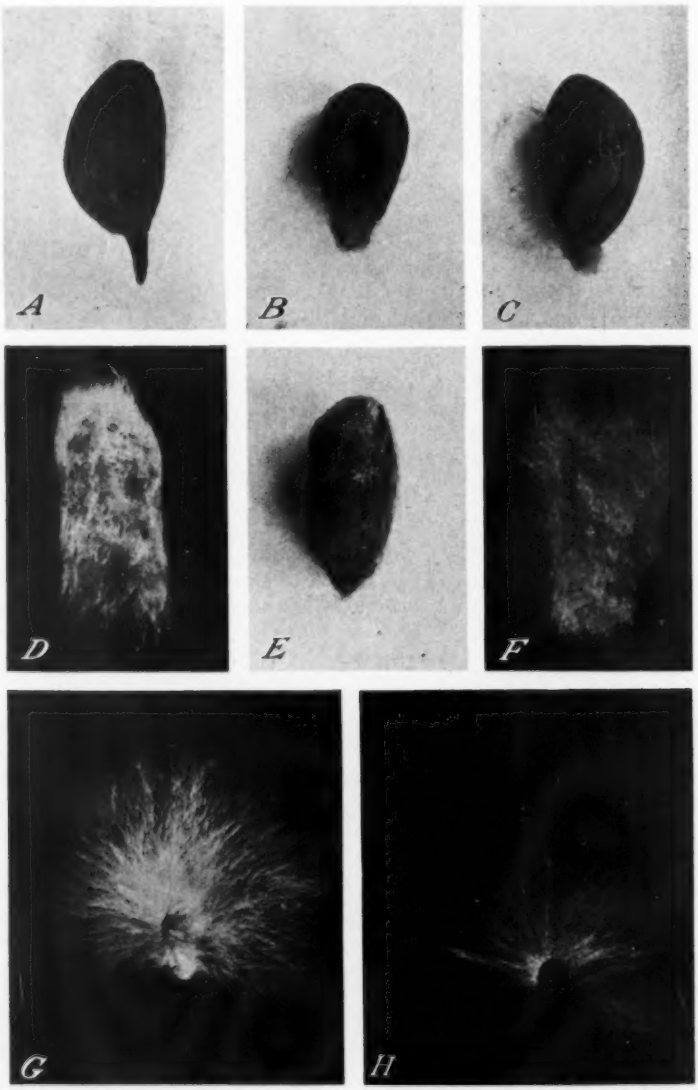

FIGURE 1.—Seed of King Naked (*A*), Cleveland Fuzzy Tip (*B*), sea island (*C*), Mexican 128–6 (*D*), Sakel × Pima (*E*), Nankeen Lint (*F*), with the lint hairs removed and the fuzz hairs combed out to show their position on the seed and relative abundance. Seed of Mexican 128–6 (*G*), King Naked (*H*), with lint hairs combed to show their distribution on the seed.

quent period. It may be seen, however, that new fibers develop in restricted numbers near the micropylar end of the ovule, but these initials serve to extend the surface range over which fibers grow rather than to contribute to the density of the fiber population of the first day. Similarly, on the third day after flowering, a few new fibers may be seen differentiating even nearer the micropylar end of the seed. Other outgrowths may continue to differentiate in this manner for several days, but the number of late fiber initials is extremely small, and in most cases the last outgrowths are seen on about the third day after flowering.

The time and place of origin of fiber initials as described here are represented diagrammatically in figure 2, *A*. In this diagram, the innermost line outlines the shape of the ovule, particularly in a submedian longitudinal section. The innermost line also represents the day of flowering and is marked 0. Each additional line to the outside represents a day after flowering up to the day of secondary wall formation, which is indicated by the outermost line. No new fibers develop after secondary wall formation has occurred. In the diagram the time and place of origin of new fibers are indicated by heavy black lines. The density of the hair population is suggested approximately by the presence of solid and broken or fragmentary lines, the solid lines representing greater numbers of hairs than the broken or fragmentary lines.

It will be noted that most of the lint hairs of the King Naked variety begin their growth on the day after flowering and that a few others differentiate during the following 2 days. Since the mature seed bears only lint hairs, it is obvious that the lint pattern is established within a period of about 3 days and that this period of lint origin falls very early in the life history of the growing ovule. Differences in fiber length may be observed from the day of fiber origin up to maturity, even in those regions of the epidermis where the fiber initials have appeared at the same time. This would seem to suggest that the length differences between mature fibers may well be due to different rates of growth.

It is interesting to note that papillate outgrowths may be observed over almost the entire surface of the ovule for 6 or 8 days after flowering, but these papillae are extremely minute and no evidence has been found that any of them subsequently elongates even as much as 5 microns.

The initiation of lint hairs in the King Naked variety is a continuous process which persists through a period of 3 or 4 days after flowering. The first hairs are formed around the chalazal end of the ovule on the day after flowering. New fibers differentiate throughout the day, and hair formation progresses from the chalazal region toward the micropylar end of the seed. By the end of the first day, half or more of the ovule may be covered by fiber initials. During the next 2 or 3 days, additional fibers are formed nearer to the micropyle and lint differentiation gradually ceases. Thus, lint hair formation progresses continuously from one end of the ovule to the other.

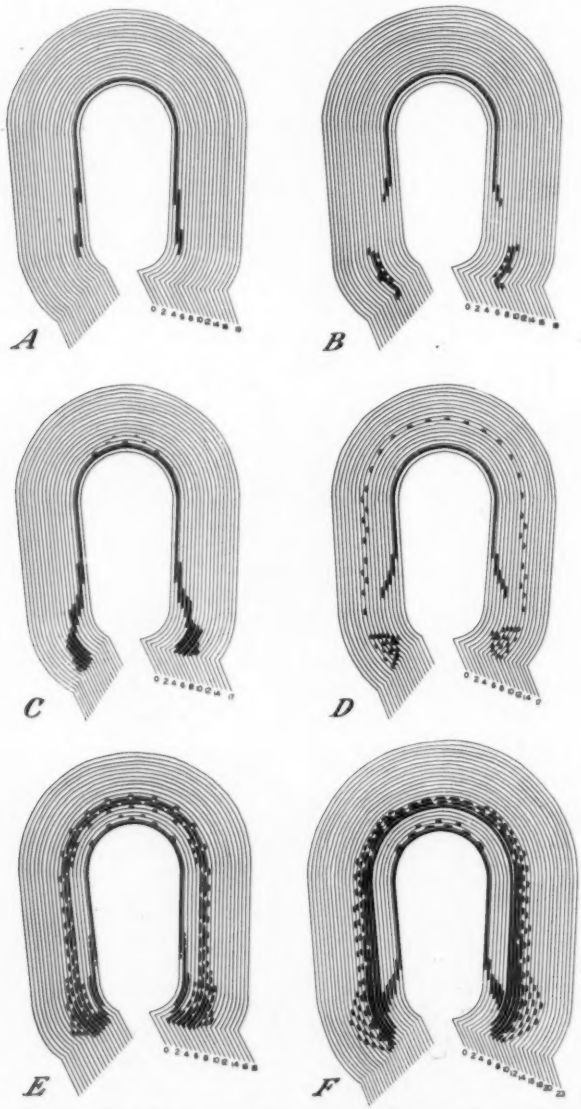

FIGURE 2.—Diagrams representing the time of origin and the distribution of the young hair initials on the surface of the ovules. The innermost line represents the surface of the ovule as seen in submedian longitudinal section on the day of flowering. Succeeding lines outside of the innermost line represent successive days after the opening of the flower. Heavy black lines indicate the position on the ovule where young hairs have been observed to originate. The relative number of hairs arising is suggested approximately by the character of the heavy black line. Where the line is solid more fibers originate than where the line is broken. The diagrams show fiber development upon ovules of varieties as follows: *A*, King Naked; *B*, sea island; *C*, Cleveland Fuzzy Tip; *D*, Sakel × Pima; *E*, Mexican 128-6; *F*, Nankeen Lint.

CLEVELAND FUZZY TIP

As the name indicates, in Cleveland Fuzzy Tip the fuzz hairs are restricted to a small area immediately surrounding the micropylar end of the seed (fig. 1, *B*). The lint hairs are usually produced over three-fourths or more of the seed surface at the chalazal end, but frequently they are limited to only about half of this portion of the seed. In this variety, therefore, both lint and fuzz hairs are present, but each hair type occupies a specific and different area on the seed coat. Although the problem is complicated in this case by the presence of both lint and fuzz, the fact that the hairs of both types are restricted to definite and separate portions of the seed coat makes it possible to determine the time and method by which each hair type originates.

On the day of flowering, the epidermis gives no evidence of fiber differentiation at any point. On the following day, outgrowths from epidermal cells of the seed coat appear in large numbers over half or more of the ovule surface at the chalazal end. As with the King Naked variety, new fibers originated for several days after flowering on portions of the ovule successively closer to the micropylar end. Unlike the King Naked variety, a few hairs were found to arise at the chalazal end of the seed on the second, third, and fourth days after flowering. The small number of these fibers is indicated by broken lines in the diagram (fig. 2, *C*).

About 6 days after flowering, proliferations from epidermal cells are formed in the region covered by fuzz in mature seeds. These outgrowths can be identified as fuzz hair initials by their position, since mature seeds bear no lint hairs in this area. As the diagram indicates, fuzz hairs continue to arise at the micropylar end of the ovule for 5 or 6 days. No hairs were observed to develop on any other portion of the ovules during this period. It seems clear, therefore, that in this variety, at least, lint and fuzz hairs originate at different times, the fuzz arising only after the complete pattern of lint hairs has been established.

The amount of lint-bearing surface in Cleveland Fuzzy Tip differs considerably from seed to seed, and this variability at maturity is reflected in a comparable variability during fiber initiation. In some instances, lint hairs differentiate only from the chalazal half of the seed, so that the final pattern is almost identical with that of sea island. In other instances, lint hairs differentiate from most of the seed surface, and since this appears to be the more common occurrence, the diagram for this variety (fig. 2, *C*) has been constructed to indicate that lint hairs arise from all of the seed surface except the fuzz-bearing portion.

MEXICAN 128–6

In the Mexican variety both lint and fuzz hairs are present over the entire surface of the mature seeds (fig. 1, *D*), so that the hair initials cannot be identified as lint or fuzz by their position on the young ovule.

On the day before flowering, a limited number of hair initials become differentiated from epidermal cells at the chalazal end of the ovule. On the day of flowering, however, hairs appear in great abundance (fig. 3, *B*) over most of the ovule surface, though few, if any, appear in the area adjacent to the micropyle (fig. 2, *E*). As in the other varieties already described, the first fibers arise at the chalazal end of the ovule, and during the same day other hairs appear

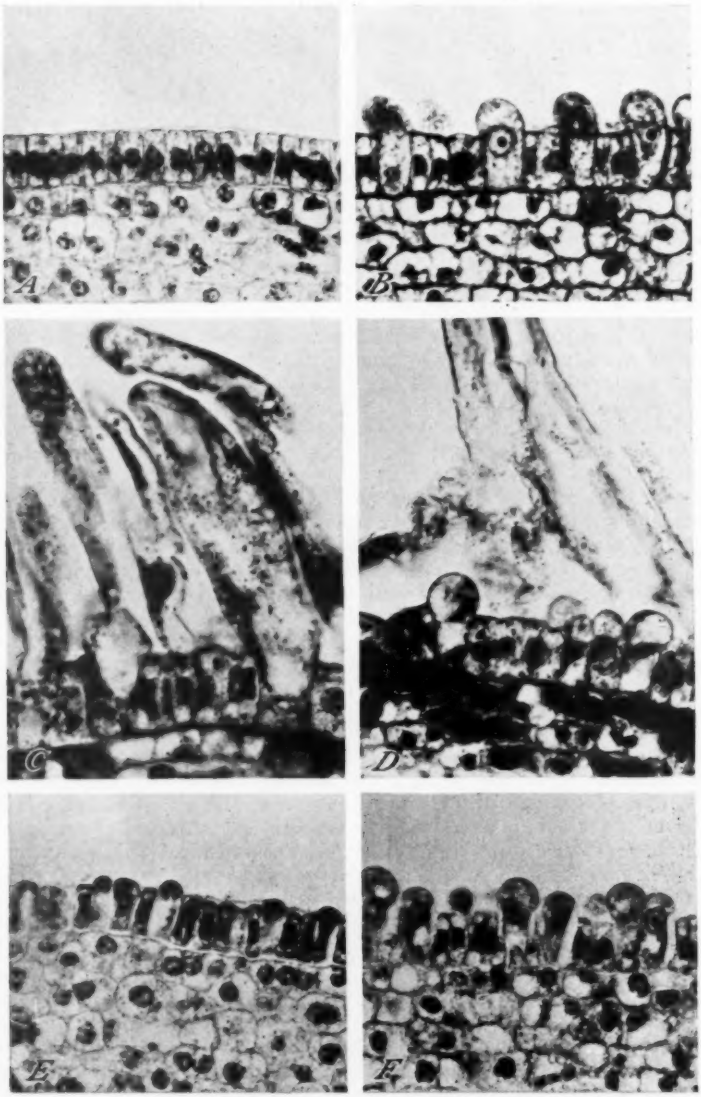

FIGURE 3.—Cross sections of epidermal layer of ovules: *A*, Mexican 128–6, the day before flowering; *B*, Mexican 128–6, the day of flowering; *C*, Mexican 128–6, 24 hours after the opening of the flower; *D*, Mexican 128–6, 5 days after flowering; *E*, sea island, 24 hours after the opening of the flower; *F*, sea island, second day after flowering.

progressively nearer the micropylar end of the ovule until it is covered with young fibers, except only the micropylar fifth or sixth of its surface area. On the day after flowering, new hairs continue to form nearer the micropylar end of the ovule and a few can be seen arising at the chalazal end as well. A small number of fibers continue to be developed from epidermal cells near the micropyle for 3 or 4 days (fig. 2, *E*). The Mexican variety differs from those previously described in having a much larger number of hair initials and also in the rapidity with which the ovule surface becomes covered with young hairs. Almost the entire surface of the young ovule bears elongating hairs within 48 hours of the day of flowering.

On the fourth or fifth day after flowering, a second outburst of fiber formation occurs (fig. 2, *E*). Fiber initials may be seen arising from newly formed epidermal cells between the bases of the first-formed hairs (fig. 3, *D*). These hair initials, like those produced on the day of flowering, first appear at the chalazal end of the ovule, and the younger hairs arise progressively nearer the micropylar end of the ovule. Within approximately 48 hours of their first appearance, almost the entire ovule surface is again covered with young hair initials. This second group of hairs continues to arise from epidermal cells of the ovule surface for a period of 4 or 5 days, and the last to be formed arise, as in previous cases, in the micropylar region. The sequence of fiber origin in the Mexican variety is shown diagrammatically in figure 2, *E*.

To interpret these observations it is necessary to recall the sequence of events described for the King Naked and the Cleveland Fuzzy Tip varieties. In these varieties it was shown that the lint hair pattern is largely established within 48 hours of the day of flowering. It was further shown that the fuzz hairs when they are present do not appear until the lint pattern had been fully determined.

A similar sequence of events apparently takes place in the Mexican variety. One set of hair initials originates within a few days of flowering, and a second set of hairs develops over the whole surface of the ovule several days later. The hairs produced on and immediately following the day of flowering correspond almost exactly in their position and time of origin to the lint hairs of the Cleveland Fuzzy Tip and King Naked varieties (fig. 2, *A* and *C*). It seems probable, therefore, that the first hair initials originating on ovules of the Mexican variety also develop into lint hairs. Following the establishment of these first-formed hairs, which are presumably the lint initials, there is an interval of several days in which very few new hairs originate.

On the fourth or fifth day after flowering, a second period of hair production is begun (fig. 2, *E*). This corresponds to the period in which the fuzz hairs develop in the Cleveland Fuzzy Tip variety. Since fuzz hairs cover the entire surface of the seed in the Mexican variety, and since the hairs arising in this second period of fiber initiation also appear over the entire surface of the ovule, it seems probable that they represent the fuzz hair initials. As will become apparent later, there is additional evidence which also indicates that the first-formed hairs develop into lint fibers and that hair initials arising several days after the opening of the flower give rise to the fuzz hairs. No significant differences in the history of hair origin and development were observed on the ovules by the three different series of Mexican studied.

NANKEEN LINT

Nankeen Lint is a variety of American upland cotton with brown lint and extremely dense fuzz. Both lint and fuzz hairs are found over the entire seed surface (fig. 1, *F*), but the fuzz is more prominent than in the other varieties studied.

As indicated in figure 2, *F*, the hairs arise in much the same manner as do those of the Mexican variety. On the day of flowering, large numbers of hairs are formed by outward extensions of the epidermal cells. Differentiation of hairs continues in regions nearer the micropylar end of the seed for 4 or 5 days after the opening of the flower. It seems highly probable that the hairs originating during the first few days after flowering give rise to lint hairs as do the King Naked and Cleveland Fuzzy Tip and the varieties of *Gossypium barbadense* that were studied. As in all fuzz-bearing varieties, the ovules of Nankeen Lint exhibit a second period of rapid hair production. This begins about 6 days after the flower opens, and active production of new hairs over the entire surface of the ovule continues for 3 or 4 days. After the tenth day relatively few new hairs are produced, and these are restricted to the micropylar end of the ovule. This prolific formation of hairs on and soon after the sixth day after flowering is exactly what would be expected, considering the varieties previously described, and suggests strongly that the fuzz hairs originate at this time. In all the varieties in which the fuzz hairs are restricted to specific areas the hairs in these regions appear about 5 or 6 days after the flower opens. This delayed appearance of hairs also occurs in Nankeen Lint, and the large number of hairs produced is clearly correlated with the dense fuzz hair population characteristic of this variety.

SEA ISLAND

Sea-island cotton, in which the lint and fuzz hairs are conspicuously restricted to specific areas on the seed, was studied as a representative of a different species of cotton (*Gossypium barbadense*). The lint is usually limited to the chalazal half or two-thirds of the seed, while the fuzz hairs form a dense growth at the extreme micropylar end (fig. 1, *C*).

On the day of flowering, some of the epidermal cells at the chalazal end of the ovule show evidence of slight protuberances. These cells are few in number and exhibit no appreciable change in size or appearance during the next 24 hours (fig. 3, *E*). On the second day after flowering, however, large numbers of hairs originate as outgrowths from epidermal cells in the chalazal half of the ovule (fig. 3, *F*). Since the papillate outgrowths observed on the day of flowering did not increase in size or number during the succeeding 24 hours, the second day after flowering is more properly regarded as the first day of fiber origin (fig. 2, *B*). On the third day after flowering, these hair initials have grown somewhat in length and new hairs may be seen arising at the micropylar margin of the zone in which hairs were formed on the second day. The area of the ovule surface on which hairs are borne is likewise increased slightly by the origin of a few new hairs on the fourth day after flowering.

Since the portion of the mature seed corresponding to the area in which these hairs are formed bears no fuzz, it is obvious that the hairs arising during the first 4 days after flowering develop into lint hairs.

On the seventh day after the opening of the flower, hairs begin to arise at the extreme micropylar end of the ovule. These hairs continue to form for 3 or 4 days. Since no lint hairs are present in this portion of the seed, these later-formed hairs must develop into fuzz hairs.

It is clear, therefore, that aside from a slower development of hair initials, sea-island cotton conforms essentially to the pattern described for the Cleveland Fuzzy Tip variety. In both cases the lint hairs appear first and the lint hair pattern is fully established upon the ovule before any of the fuzz hairs originate.

SAKEL × PIMA

The mature seeds of Sakel × Pima have both lint and fuzz hairs. The lint pattern is very similar to that of sea island and Cleveland Fuzzy Tip. The fuzz hair pattern is very different, however, for fuzz occurs irregularly over the entire surface of the seed. The fuzz hairs at the micropylar end are much like those of sea island, but on the rest of the seed the fuzz is very short and brown, adheres closely to the seed coat (fig. 1, *E*), and contains tanniferous substances.

In Sakel × Pima, hairs were observed to originate from epidermal cells on the chalazal half of the ovule on the day after the opening of the flower. New hairs continue to form during the next 3 or 4 days, but they are restricted to a small zone at the margin of the area upon which hairs were initiated during the first day after the opening of the flower (fig. 2, *D*). For reasons that will become apparent later, these first-formed hairs very probably develop into the lint hairs.

A few days after the flower opens, the epidermal cells of the seed coat become altered in appearance. This alteration appears to be due to the accumulation of mucilaginous and tanninlike compounds in the cell vacuoles. This accumulation seems to bear no relation to the age of the individual cells of the epidermal layer, for these substances are present in newly divided cells as well as in cells that are several days old. The presence of these substances in the vacuoles of the hairs is evidence that such hairs originated several days after the opening of the flower. Hairs produced 24 or 48 hours after flowering apparently do not contain these substances. Since the lint hairs of this variety are free from such mucilaginous and tanniferous deposits, it seems clear that they originated during the first few days after the flower opened.

Five or six days after flowering, hair initials appear near the micropyle, and they continue to arise for several days (fig. 2, *D*). This is the portion of the seed that bears conspicuous fuzz hairs and no lint, so it is obvious that the hair initials in this position develop into fuzz. Occasional epidermal cells on the chalazal half of the ovule begin to produce hairs on about the eighth day after flowering. These first appear near the chalazal end and arise successively nearer to the micropylar end of the seed (fig. 2, *D*). These hairs contain the mucilaginous and tanniferous deposits generally present in the epidermal layer and so can be identified with reasonable certainty as fuzz initials, since the mature fuzz hairs of this variety contain these substances, whereas they are absent from lint hairs.

In Sakel × Pima the evidence suggests clearly that the lint hairs are formed within a few days of the opening of the flower and that the

lint hair pattern is fully determined before the fuzz hairs appear. The fuzz hairs first originate about 5 days after flowering in the micropylar regions, but some 8 or 9 days after blossoming additional fuzz hairs are initiated upon the remaining surface of the ovule.

DISCUSSION

From the data that have been obtained in this study it is possible to draw certain definite conclusions and to formulate a hypothesis relative to the origin of lint and fuzz hairs. Before examining the theoretical considerations it will be advisable to set forth briefly the facts that are known.

In all the ovules examined in this study, with the single exception of the King Naked variety, on which lint hairs alone are present, young hairs were observed to originate at two different periods. The first period was on or very soon after the day of flowering, and the second period usually began 5 or 6 days after the opening of the flower. In all those varieties in which the lint hairs are present only upon restricted areas of the mature seeds, and in which it is therefore possible to identify the lint hair initials beyond question, nearly all the lint hairs were found to originate within 2 or 3 days after the opening of the flower. Few if any new lint hairs are initiated in these varieties later than 5 days after the opening of the flower. In those varieties where fuzz hairs are restricted to areas devoid of lint hairs and where the fuzz hair initials can therefore be identified with certainty, the fuzz begins to develop only after the lint pattern has been completely established. This means that the fuzz hairs originate in a period from 5 to 10 days after the flower has opened.

With the exception of Sakel × Pima, the time of lint and fuzz origin cannot be determined with certainty in the varieties that have both lint and fuzz hairs over the entire surface of the seed. The two varieties that have a dense fuzz population over the seed surface (Mexican and Nankeen Lint) exhibit many aspects of hair development that appear similar to those of the pattern types. There is, in other words, a very real analogy between their behavior in the matter of hair origin and the behavior of the varieties in which lint and fuzz can be identified by the position of the hair initials in the ovule. In such varieties or types as King Naked, Cleveland Fuzzy Tip, sea island, and Sakel × Pima, where the lint hair initials can be identified without question, they have been found to originate on or soon after the flower opens. A similar phenomenon occurs in the Mexican and Nankeen Lint varieties. By analogy, it might be assumed that these first hair initials give rise to the lint hairs. In Cleveland Fuzzy Tip, sea island, and Sakel × Pima, where the fuzz hair initials can be identified clearly, the fuzz hairs are found to originate from 6 to 10 days after flowering and well after the lint hair pattern is established. Similarly, in the Mexican and Nankeen varieties a number of hairs are observed to originate from 5 to 10 days after flowering and well after the pattern of the first-formed hairs has been determined. By analogy, these might be regarded as the fuzz hair initials. Analogies, however, are uncertain foundations upon which to erect trustworthy conclusions. It is possible, even probable, that the first set of hair initials in the Mexican and Nankeen Lint varieties is the lint hair initials, and that the second set, as in the other varieties, represents

the fuzz hair initials. In the absence of further evidence, however, it would not be possible to assert positively that the first-formed hairs produce the lint and that the second set of hair initials gives rise to the fuzz in these varieties.

Fortunately, there is evidence of a different sort which strengthens the assumption that the lint hairs develop from the hairs formed soon after the opening of the flower and that the fuzz hairs originate from the hair initials that appear 5 or more days after flowering. The statistical studies of Turner (*9*), for example, show that there is a decided increase in the total number of hairs on the cotton ovules as they grow older. One-day-old ovules of the Nandyal 14 variety were found to possess some 4,530 hairs, while 28-day ovules had 16,420 hairs. Likewise, 1-day-old ovules of the Punjab-American 4–F variety were estimated to have 5,980 hairs, in contrast to the 25,090 hairs counted upon the 28-day-old ovules. These data become highly suggestive when considered in the light of the number of lint hairs found upon mature seeds of these varieties. Nandyal 14 was found to have only 4,000 lint hairs at maturity, and Punjab-American 4–F had approximately 7,900. The number of lint hairs on mature seed, therefore, is not very different from the number of hairs found upon 1-day ovules. This is exactly what would be expected from the results of the present study, since wherever lint hairs could be positively identified at the time of their origin they were found to arise within a day or two of the day of flowering. The increase in the number of hairs upon the older ovules that is reported by Turner can be explained by pointing out that the fuzz hairs, wherever they could be positively identified at the time of their origin, have been found to originate in a period between 5 and 10 or more days after the opening of the flower. The data obtained by Turner from a purely statistical study harmonize very well with the results of the cytological study here reported.

Another line of evidence of a very different kind that supports the hypothesis of early origin of the lint and later origin of the fuzz is that reported by Harrison (*7*) as a result of his studies of metaxenia in cotton. Harrison found that hybrids between inbred Pima and Hopi strains showed definite evidence of metaxenia. One of these experiments is of interest in relation to the problem of lint and fuzz origin. Harrison reported that when Hopi pollen was used upon Pima the number of lint hairs produced upon the resulting seeds was not different from those upon inbred Pima seeds but the amount of fuzz was appreciably reduced. These results harmonize nicely with those of the present study. If lint hairs originate within 24 or 48 hours of the time of flower opening they could hardly be influenced by a hybrid embryo. Fertilization does not occur until after the lint hair pattern has been established. Fuzz hairs, however, presumably originate after fertilization has been accomplished, and the density of the fuzz pattern could, therefore, be influenced by the hybrid embryo. The results of Harrison's work may be interpreted as supporting the thesis that fuzz hairs arise after the lint pattern has been fully determined.

It seems clear from the foregoing discussion that there is considerable evidence in support of the hypothesis that lint hairs arise on or very soon after the day of flowering, while the fuzz hairs do not appear until 5 or more days after the flower opens. This is known to be true of those varieties studied in which the lint and fuzz initials could be identified by their position on the ovules. The history of hair origin

in the varieties studied in which lint and fuzz hairs could not be identified by position or appearance at the time of their origin is thoroughly consistent with early development of lint and later origin of fuzz. The early appearance of lint hairs is further suggested by statistical studies of hair density at different ages and by experiments designed to demonstrate metaxenia in cotton. The thesis that lint hairs arise early and that fuzz hairs originate only after the lint hair population has been fully determined seems, therefore, to be justified by the evidence presented.

SUMMARY

The time and place of origin of lint and fuzz hairs on the ovules of six selected varieties of cotton have been studied. The hairs in all varieties were found to have an essentially similar developmental history.

In the King Naked variety most of the lint fibers originate on the day of flowering and additional lint may differentiate during the next 2, 3, or 4 days.

In Cleveland Fuzzy Tip, lint initials differentiate in abundance on the day after flowering and others make their appearance in the following 3 or 4 days. Fuzz fibers originate continuously at the micropylar end of the ovule from about the sixth to the eleventh day.

In Mexican 128–6, hairs that are presumably the lint initials appear in abundance on the day of flowering and during the next 3 or 4 days in areas near the micropylar end of the ovule. Hairs that presumably give rise to fuzz originate on about the fourth day after flowering and continue to arise for a period of 3 or 4 days.

In Nankeen Lint, hairs believed to be the lint initials differentiate from the epidermal cells on the day of flowering and continue to form in the region of the micropyle for the next 4 or 5 days. Hairs believed to be the fuzz initials first appear in abundance on the sixth day after flowering and continue to originate for a period of about 5 or more days.

In sea island, almost all of the lint fibers originate on the second day after flowering, but a small number of others may arise on the following 2 or 3 days. The fuzz initials arise on about the seventh, eighth, and ninth days after flowering.

In Sakel × Pima, hairs that are presumably the lint initials appear on the day after flowering, and a few others develop during the next 3 or 4 days. The fuzz at the micropylar end of the seed originates between the fifth and tenth days after flowering. The fuzz on other portions of the seed apparently begins to develop on about the eighth day after the flower opens.

It is suggested that lint hairs originate when or soon after the cotton flower opens, and that the first fuzz hairs appear only after the lint population has been fully determined. It is further shown that this thesis is consistent with the results of other investigators.

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ROGAS UNICOLOR (WESM.), A BRACONID PARASITE OF THE SATIN MOTH[1] [2]

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INTRODUCTION

Rogas unicolor (Wesm.) is a European species parasitic on the satin moth (*Stilpnotia salicis* L.). It was first reared by workers of the Bureau of Entomology, United States Department of Agriculture, at Budapest, Hungary, in 1927. At that time only two adults were recovered, but in 1932 about 250 cocoons were obtained by rearing, and in 1933 and 1934 more than 7,000 cocoons were sent to the United States. Several colonies of adults have been liberated in areas infested with the satin moth in New England and Washington. Although the species is not known to have become definitely established, numerous observations have been made in the laboratory regarding its biology and morphology, and this paper gives the results of these observations.

REVIEW OF LITERATURE

Although the genus *Rogas* includes many species that are parasitic on common lepidopterous pests throughout the world, notes on their biology and habits are scattered and incomplete. *Rogas unicolor* was mentioned briefly as a parasite of *Stilpnotia salicis* in Hungary by Brown (*1*) [4] in 1931. De Fluiter (*4*) listed *Petalodes* (*Rogas*) *unicolor* (Wesm.) [5] as a parasite of *S. salicis* in Holland in 1933, giving a list of secondary parasites reared from the cocoons. *Petalodes unicolor* (Wesm.) and *Rogas unicolor* (Wesm.) are distinct, but apparently *Rogas unicolor* is the species that De Fluiter reared from the satin moth. Dustan (*2*) gave an excellent account of the internal anatomy of the full-grown larva of *Rogas hyphantriae* Gahan, which has been very helpful in this study. Fiske (*3*) gave brief notes on what he called "*Rhogas intermedias* Cresson" [5] as a parasite of *Clisiocampa* (=*Malacosoma*) *americana* F., emphasizing the role played by its hyperparasites. This species, however, is an undescribed one which was obviously misidentified. Pennington (*7*) briefly discussed the life history of *Rogas terminalis* (Cress.), parasite of *Cirphis unipuncta* Haw., and noted the production of males in parthenogenesis. Hyslop (*6*) noted *Rogas autographae* Vier. as a parasite of *Autographa gamma* L. (*californica* Speyer) and described cocoon formation. Tothill (*9*) gave a few notes on *Rogas* sp. as a parasite of *Hyphantria cunea*

[1] Received for publication Oct. 23, 1937; issued May 1938.

[2] This study was conducted under the direction of C. W. Collins at Melrose Highlands, Mass., in 1932, 1933, and 1934, and under the direction of R. C. Brown at Melrose Highlands, Mass., and New Haven, Conn., in 1935.

[3] The writer wishes to express his gratitude to W. F. Sellers for collections and notes made at Budapest, Hungary; to Karoly Mihalyi for field collections; and to Mary F. Benson for the drawing of the adult.

[4] Reference is made by number (italic) to Literature Cited p. 535.

[5] The writer is indebted to C. F. W. Muesebeck, of the Division Insect of Identification, for these notes regarding identifications.

(Drury). Husain and Mathur (*5*) briefly noted the life history of "*Rogas testaceus* Grav. var.",[5] parasite of *Earias insulana* Boisd. and *E. fabia* Stoll. Apparently *R. testaceus* (Spin.) was intended, for Gravenhost did not describe a species of *Rogas* under this name. Hyslop (*6*), Tothill (*9, pl. 3*), and Pennington (*7*) gave good figures of the adults and cocoons of the species they worked with, and Pennington also gave a figure of the egg.

ECONOMIC IMPORTANCE

Rogas unicolor has been recorded from Belgium, Holland, Germany, and Hungary, and it seems probable that it is present in many of the places where *Stilpnotia salicis* occurs in Europe. So far as is known, it has been reared only from this host. Apparently it is of slight economic importance. De Fluiter (*4*) notes recovering it from *S. salicis* in Holland, but he does not give the number of cocoons obtained. The Bureau of Entomology conducted large-scale rearing work at several points in Austria and Hungary from 1927 through 1934 for the recovery of parasites of *S. salicis*, but *R. unicolor* was never recorded in appreciable numbers except at Budapest, Hungary. At that point from 1929 to 1934 there was a light infestation of the satin moth on a sand bar in the Danube River. This sand bar, about 1½ miles long and from 100 to 200 yards wide, supports a bushy growth of poplar ranging in height from 3 to 8 feet. In 1931, 11 cocoons were reared from 6,000 larvae. In 1932, 286 cocoons were recovered from collections totaling 26,980 larvae. In 1933, 364 cocoons were obtained from collections of 35,950 larvae, and 1,890 cocoons were collected in the field. In 1934 rearing work was discontinued, but 5,065 *R. unicolor* cocoons were collected in the field for shipment to the United States, and it was estimated that, if funds had been available, from 2,000 to 3,000 more might have been brought together.

In spite of the low percentage of parasitization by *Rogas*, the field collections showed that, barring very high secondary attack, there were enough cocoons in the field to supply a large number of adults. It was therefore felt that possibly under somewhat different conditions, such as might exist in the United States, the species could have an excellent chance of becoming more effective.

It should be pointed out at this time that cocoon collection in the field was greatly facilitated by the fact that the parasitized host larva spins a conspicuous white web, inside of which the parasite cocoon is formed.

INTRODUCTION INTO THE UNITED STATES

There were 256 *Rogas unicolor* cocoons in the first shipment sent to the United States in 1932. Out of 225 adults that emerged, 224 were females. There seemed a strong possibility, therefore, that this species normally produced females in parthenogenesis; so only females were liberated. This possibility was later confirmed, and all liberations have consisted entirely of unmated females. The number and extent of colonizations is shown in table 1.

Unfortunately for this work, during the period when liberations were made in New England the satin moth was on the decline and, although good infestations were present at the time of colonization, they practically disappeared in the following year. In Washington conditions

were far better for colonization, for a general infestation has persisted throughout the area. The adult parasite has never been recovered in the United States, although in 1934, the winter following liberations, a first-instar *Rogas* larva was dissected from a hibernating satin moth larva collected at the colonization point in Portland, Maine.

TABLE 1.—*Colonization of Rogas unicolor in the United States*

Point of liberation	Year	Females shipped	Living females liberated
		Number	*Number*
Portland, Maine	1933	500	500
Topsham, Maine	1933	500	500
South Harpswell, Maine	1934	500	500
Concord, N. H.	1933	150	150
Taunton, Mass.	1932	200	200
South Yarmouth, Mass.	1933	500	500
Woonsocket, R. I.	1934	500	500
New Haven, Conn.	1934	300	293
Olympia, Wash.	1934	432	430
Bellingham, Wash.	1934	500	497
Stanwood, Wash.	1934	500	457
Kent, Wash.	1934	500	492
Total		5,082	5,019

DESCRIPTIONS

THE ADULT

The adult (fig. 1) was described by Wesmael (*12*) in 1838 as a species of the genus *Aleiodes*. In 1863 Reinhard (*8*) placed it under Nees' genus *Rogas*.

THE EGG

The newly laid egg (fig. 2, *A*) measures 0.40 mm long and 0.11 mm wide. It is very slightly curved, bluntly rounded at the ends, and slightly broader at the cephalic extremity. The chorion is smooth and hyaline except for a slightly roughened area at the cephalic end. A conspicuous round, light area may be observed near the caudal end of the embryo. This is the gonad of the first-instar larva.

THE LARVA

There are five larval instars. The first and the last are distinct, but the intermediate instars differ little from one another except in size and mandibular measurements. Each of the first four instars has an extremely delicate skin, and often the next larval instar may be distinguished as it develops under the skin about to be molted.

FIRST INSTAR

The first-instar larva (fig. 2, *B*) averages 0.40 mm long and 0.12 mm wide when hatched. It attains a size of about 0.60 mm long and 0.15 mm wide preparatory to hibernation, and in the spring develops to almost three times this size before molting to the second instar. It possesses a head and 13 well-differentiated segments. The cuticle is smooth, without spines or setae, and there is no tracheal system. The head (fig. 2, *C*) has a well-developed hypostoma and pleurostoma.[6] The epistoma, instead of constituting a continuous arch

[6] The terminology of the head structures is that of Vance and Smith (*10*).

above the mouth opening, is represented by two well-developed struts which run vertically above the superior pleurostomal rami. The maxillae and labium are defined by lightly sclerotized markings, bearing four pairs of setae. There are four sensoria just above the mouth opening. The mandibles (fig. 2, *D*) are simple, sharply curved hooks, measuring 0.02 mm. The internal anatomy is similar to that of the full-grown larva, which will be described fully, except that in the first instar the gonads are well developed and conspicuous. They are circular in outline and occupy a large part of segments 10 and 11 above the hind-intestine.

Second Instar

Second-instar larvae range from 1.8 mm long and 0.56 mm wide to 3.75 mm long and 0.95 mm wide. The tracheal system is developed, and,

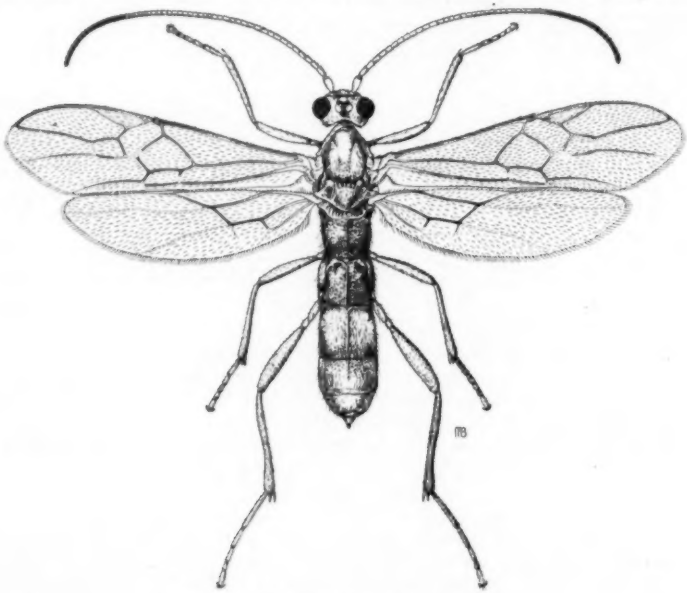

FIGURE 1.—*Rogas unicolor*. Adult female, × 9.

although there are no spiracles, there are spiracular stubs in the second and third thoracic and first eight abdominal segments. The head structures are weakly sclerotized. The hypostoma is well defined, but the mandibular and maxillary lobes are weakly shown and the epistoma forms a barely discernible arch over the mouth. The mandibles have the same general form as those of the full-grown larva. They measure 0.04 mm. The gonads are inconspicuous.

Third Instar

Third-instar larvae exhibit but slight differences from those of the preceding instar. They range from 4.0 mm long and 1.0 mm wide to 5.0 mm long and 1.32 mm wide. The mandibles measure 0.06 mm.

Fourth Instar

Fourth-instar larvae range from 5.0 mm long and 1.5 mm wide to 9.0 mm long and 2.0 mm wide. There are still no open spiracles, the integument is bare, and except for the hypostoma and pleurostoma

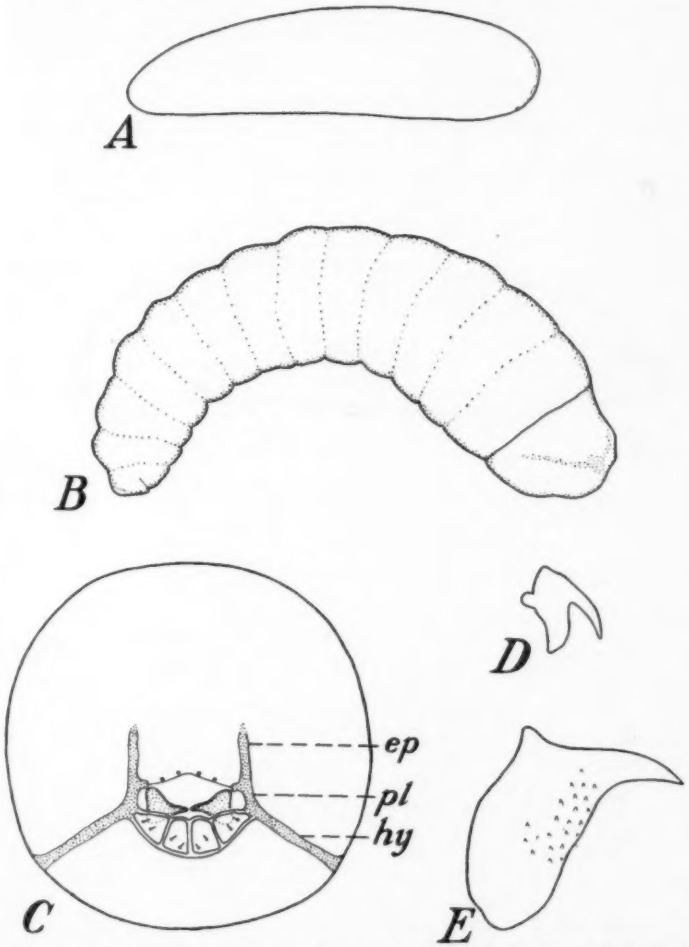

FIGURE 2.—*Rogas unicolor*: *A*, Egg, × 152. *B*, First-instar larva, lateral view, × 150. *C*, Head of first-instar larva, front view; *ep*, epistoma; *pl*, pleurostoma; *hy*, hypostoma, × 458. *D*, First-instar mandible, × 650. *E*, Last-instar mandible, × 235.

the head skeleton is practically undefined. The mandibles measure 0.10 mm.

Fifth Instar

The full-grown larva (fig. 3, *A*) measures about 10 mm long and 2.5 mm wide. It is yellowish white, and when quiet assumes the char-

acteristic shape, with head curved downward, as shown in the figure. The first eight abdominal segments bear prominent lateral folds. The integument bears tiny cuticular spines as indicated. The last abdominal segment bears, in addition, several (about 12) pairs of sensory setae. Sensory setae are also present on the other segments,

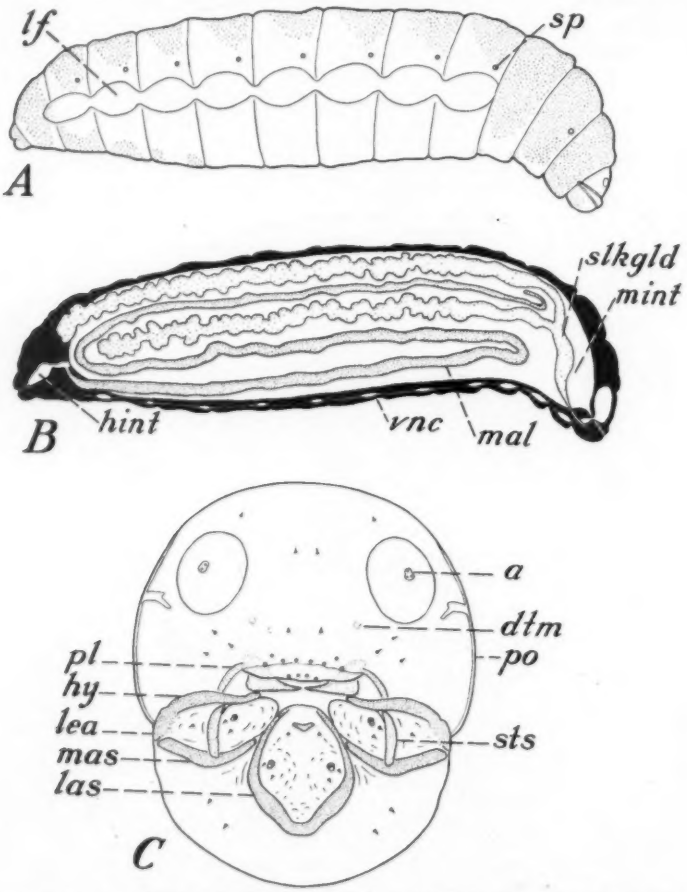

FIGURE 3.—*Rogas unicolor*: *A*, Full-grown larva; *sp*, spiracle; *lf*, lateral fold, × 11. *B*, Full-grown larva, internal anatomy; *slkgld*, silk gland; *mint*, midintestine; *mal*, malpighian tube; *vnc*, ventral nerve cord; *hint*, hindintestine, × 11. *C*, Head of full-grown larva, front view; *a*, antenna; *dtm*, dorsal tentorial mark; *po*, postocciput; *sts*, stipital sclerome; *pl*, pleurostoma; *hy*, hypostoma; *lea*, lateral epicranial angle; *mas*, maxillary sclerome; *las*, labial sclerome, × 72.

but owing to similarity with the cuticular spines and unevenness of distribution their exact number and location were not determined.

The head, seen from the front (fig. 3, *C*), presents well-defined characters. The antennal rudiments are somewhat darkened areas containing two or three minute papillae. They are surrounded by

large oval areas, the antennal foramina. The postocciput is well defined laterally. Just below the antennal foramina pronounced grooves extend inward from the postocciput. These grooves may be the temporal fossae. The hypostoma widens and divides at the lateral epicranial angles, where one side is fused to the strong transverse tentorial bar. The stipital sclerome is apparently not attached to the hypostoma. It sets off the heavy fold of the maxillary region. The maxillary and labial palpi each have two slightly raised round sensoria. The mandibles (fig. 2, *E*) are simple and broadly oval at the base. On the inner side of the basal part they bear a number of minute spines. They measure 0.14 mm. An area on the ventral portion of the head is armed with cuticular spines.

The tracheal system is composed of a pair of lateral trunks, which give rise to a main dorsal and a main ventral branch in each segment except the head and the last segment. There are nine pairs of spiracles. A pair occurs on the mesothoracic segment and a pair on each of the first eight abdominal segments. They are joined to the longitudinal trunks by short spiracular branches. A spiracular stub occurs in the metathoracic segment, but there is no spiracle. The mesothoracic spiracle measures 0.04 mm in diameter. The main dorsal branches in the first segment are connected, and in each of the first eight abdominal segments small tracheae arising from the main ventral branches unite to form a ventral transverse commissure. The head and the last segment receive branches from the main longitudinal trunks. The main dorsal and ventral branches give off numerous small tracheae, which branch profusely. Some small tracheae also arise from the longitudinal trunks.

The digestive system (fig. 3, *B*) is similar to that described by Dustan (*2*) for *Rogas hyphantriae* Gahan, although larvae of *R. unicolor* were not sectioned, and many of the details described for *R. hyphantriae* were not studied.

The alimentary canal consists of a short, slender foreintestine, a large midintestine, occupying most of the body cavity from the first to the eighth abdominal segment, and a short hindintestine. The contents of the midintestine are brownish pink. The hindintestine is composed of three well-defined areas—a short bulbous section just behind the midintestine, an even shorter constricted section, and a large saclike rectum. The malpighian tubes are attached to the first section. The rectum is about three times as wide as it is high. The salivary glands are well-developed tubes lying on each side of the body. They unite in a short, common duct, which has a U-shaped opening on the floor of the mouth. The common duct divides into two tubes at the posterior border of the head. Each tube extends upward to the third segment, where it bifurcates. Each branch possesses many actively secreting cells that are grouped in conspicuous oval nodules joined along the main tube. Posteriorly these nodules are more numerous, and consequently the tubes become considerably widened. There is only one pair of malpighian tubes, but they are exceedingly long and are attached very closely to one another at the base of the hindintestine. They do not have a common lumen as in *Rogas hyphantriae*. They separate almost immediately, running just above the ventral nerve cord to the third thoracic segment, where they turn, run posteriorly almost as far as the hind intestine, turn again, and run

anteriorly almost to the second thoracic segment, where there is another short turn before they end. They are for the most part straight-sided tubes, but here and there a slight swelling occurs.

The brain and the nerve cord are distinct. The ventral nerve chain includes 11 double ganglia, the terminal one being in the eighth abdominal segment. The abdominal ganglia are fused. Each is located at the anterior border of the segment, and gives off a pair of long nerve fibers running posteriorly and laterally through the segment. The terminal ganglion is evidently a fusion of the last three segments, for it sends out three pairs of nerves.

The heart, or dorsal blood vessel, may be distinguished readily in living specimens. It runs from the first segment just behind the brain abruptly dorsad to the median line, and along this line to the twelfth segment.

The urate cells are conspicuous just beneath the cuticle. They are irregular in shape, semiopaque, and occur from the second to the seventh abdominal segments, inclusive.

The histoblasts of the various external organs are easily distinguishable, since they lie just underneath the cuticle and are semiopaque. The antennal histoblasts are situated behind the antennal rudiments; those of the legs are on the venter of the thoracic segments; those of the wings are found laterally on the second and third thoracic segments; and the histoblasts of the female genitalia are located ventrally in the eighth and ninth abdominal segments.

The rudiments of the ovaries are located in the seventh abdominal segment. They consist of two spindle-shaped bodies with long, narrow stalks ventrally and numerous tiny filaments dorsally. The stalks are attached to the ventral wall on each side of the ventral nerve chain, and the filaments are joined dorsally.

THE COCOON AND PUPA

Characteristic of the genus, the cocoon of *Rogas unicolor* is formed inside the dead host larva's skin. The contents of the host larva are almost entirely consumed, and the parasite spins a light, but tough, brown cocoon within the host skin. As the skin dries it forms a dry, taut case, conforming closely to the shape of the full-grown parasite larva. The head of the dead host larva remains attached to the skin and is curved downward.

The pupa is of the usual hymenopterous type. It is oriented toward the caudal end of the host larva.

BIOLOGY

Rogas unicolor has one generation a year. It passes the winter as a first-instar larva within the hibernating host larva. Development proceeds slowly in the spring, and the parasite larva does not become full grown until the host larva is in the penultimate instar. Under insectary conditions in New England on an average 12 days is spent in a cocoon. *Rogas* adults issue from June 21 to July 7, the majority appearing the end of June. Probably issuance would be somewhat later under field conditions. Satin moth eggs are deposited late in June and in July. They hatch in about 15 days, and 5 to 6 days are spent in the first instar. *Rogas* females attack the second-instar larvae. Females issuing the first of July probably do not find an

abundant supply of suitable host material for 2 or 3 weeks, but undoubtedly some larvae are present soon after they appear.

As in other species of *Rogas*, the adults issue by gnawing an irregular hole through the dorsal side of the cocoon near the caudal extremity of the dead host's larval skin. In one or two instances the emergence hole has been found on the venter. The adults are rather inactive, slow-moving insects. At the laboratory they were confined in glass-topped wooden boxes and fed dry lump sugar and a honey solution (1 part honey to 5 parts water) held on absorbent cotton or sponges. When placed in a cool, dark room they lived on an average 34 days. When placed in 8-inch glass vials for continuous reproductive work, they lived a somewhat shorter time. Six females used in reproduction-capacity work in 1933 lived on an average 22 days, with a minimum of 12 and a maximum of 32 days. In 1934 the females used in similar work were held for 3 to 4 weeks before suitable host larvae could be provided, and they lived on an average only 8 days after beginning oviposition.

As already indicated, experiments have shown that *Rogas unicolor* normally produces females by parthenogenesis. A number of larvae were attacked at the laboratory by both mated and unmated females. In all 90 adults were reared, and 89 of them were females. The one male was the progeny of an unfertilized female. Apparently about the same proportion of females are produced under natural conditions, for during a 3-year period out of 5,492 adult *Rogas* issuing from cocoons received from Europe only 40 were males.

Although females are normally produced in parthenogenesis, the sexes mate fairly readily. Eight pairs averaged 30 seconds in coitu.

Rogas females oviposit very readily in second-instar host larvae. First-instar larvae are so small that the ovipositor cannot be successfully inserted. When a *Rogas* female comes near a host larva, she quickly touches it with her antennae and moves toward it with abdomen curved downward ready to strike. If the larva stops, she often stands still over it a moment and then prods it gently with her abdomen. When the larva is moving, she inserts her ovipositor by a quick forward thrust of the abdomen. Oviposition requires about a second, and the parasite then hurries away. Since the parasite's ovipositor is very short and the host larva small, the larva is often lifted up, and frequently rolls itself into a tight ball. If the larva rolls up before the ovipositor is actually inserted, the parasite may make use of her long antennae and palpi to hold it in position for attack. The parasite shows no discrimination, frequently attacking the same host larva a number of times, but she always walks away a short distance after each egg is laid, and is therefore not immediately attracted to the same larva. Observations conducted during two seasons on isolated females indicated that *Rogas unicolor* has a rather high reproductive capacity. The maximum number of eggs laid by one female was 322, but when this female was dissected there were still a number of eggs in her ovaries and ovarioles. Each ovary has two very long ovarioles, and it is very difficult to count all the eggs present. This particular female had at least 100 more eggs in the process of formation, and it is quite possible that more might have been developed. The maximum number of eggs laid by a single female over a 2-day period was 88.

When it is first deposited, the egg apparently floats freely in the body cavity of the host larva, for it floats out very readily upon dissection. After about 24 hours, however, it seems to be lightly attached to various host organs, but most frequently to the silk glands, malpighian tubes, or fat body. The egg hatches in about 53 hours. The tiny parasite larva floats freely in the body cavity of the host. It ingests a small quantity of the host body fluids, increasing slightly in size before hibernation. In the spring it develops very slowly, first-instar larvae having been dissected out as late as June. The next three instars are completed within 3 or 4 days, and it is difficult to find a larva of an intermediate instar without traces of the next one already apparent through the thin skin. Several days are spent in the last larval instar. The contents of the host larva are almost entirely consumed and then the parasite cocoon is spun. During the greater part of this instar the head of the parasite is oriented similarly to that of the host. As the cocoon is spun the parasite reverses its position completely, and pupation takes place with the head of the parasite toward the posterior segments of the host and its venter toward the host dorsum. Upon completion of the cocoon the larva voids its meconium and pupation takes place within about 24 hours.

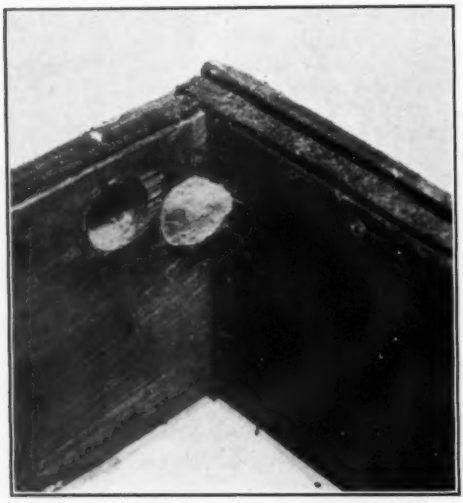

FIGURE 4.—Web spun by *Stilpnotia salicis* larva parasitized by *Rogas unicolor*.

Vickery (*11*) found that just before cocoon formation the larva of *Rogas laphygmae* Vier. cuts through the skin and pushes the fluids and material it does not eat out of the host larva's body. The drying of these fluids attaches the cocoon to the plant on which it is resting. Fiske (*3*) reported that larvae of "*Rhogas intermedias* Cresson" attach their cocoons with a bit of brown silk extruded from a puncture on the ventral thoracic surface. *Rogas unicolor* does not cut through the host larval skin. The cocoons are dry and unattached.

EFFECT OF PARASITE ON HOST

The growth of *Stilpnotia salicis* larvae parasitized by *Rogas unicolor* is considerably retarded. There is no apparent difference in parasitized and unparasitized larvae until the penultimate instar. Those that are unparasitized develop rapidly through the last instar and begin to pupate, while those that are parasitized fail to molt to the last instar and become very sluggish. Finally, about the time the parasite enters the fourth instar, the caterpillar seeks a sheltered

place and spins a dense, white web that is usually oval (fig. 4). In the field this web is usually spun between leaves. The parasite cocoon is formed inside this protective covering.

HYPERPARASITES

Unfortunately *Rogas unicolor* is attacked by a number of hyperparasites. Fourteen species have been reared from cocoons collected from the field at Budapest, and De Fluiter (*4*) records four species reared from "*Petalodes* (*Rhogas*) *unicolor* (Wesm.)" in Holland. *Dibrachys cavus* (Walk.) was the most important hyperparasite at Budapest. During 1933 and 1934 it parasitized more individuals than all the other species together. It is gregarious, and during the 2-year period on an average 15 adults were reared from each *Rogas* cocoon. This species is present in New England, and Fiske (*3*) has recorded *Dibrachys boucheanus* (=*cavus* Walk.) as an important parasite of "*Rhogas intermedias* (Cresson)."

Data concerning the hyperparasites reared from *Rogas unicolor* cocoons collected at Budapest have been summarized in table 2.

In 1933 hyperparasites issued from 14 percent of the cocoons that produced adults, and in 1934 from 17 percent. Since this material was collected in the field, it gives some indication of natural conditions, but probably a much higher percentage would have been destroyed if the cocoons had been left there until the adults emerged.

TABLE 2.—*Parasitization of field-collected cocoons of Rogas unicolor from Budapest, Hungary, in 1933 and 1934*

Species of parasite [1]	1933			1934		
	Parasitized cocoons	Parasites issued	Average parasites per cocoon	Parasitized cocoons	Parasites issued	Average parasites per cocoon
Itoplectis scanica Vill.	25	25	1	98	98	1
Theronia atalantae Poda	19	19	1	65	65	1
Hemiteles areator Panz.	2	2	1	6	6	1
Mesochorus tuberculiger Thoms.	34	34	1	65	65	1
Mesochorus pallidus Brischke	1	1	1			
Brachymeria intermedia (Nees)	2	2	1	12	12	1
Catolaccus ater Ratz.	9	9	1	4	4	1
Monodontomerus dentipes Boh.	4	12	3	6	20	3+
Tetrastichus rapo Walk.	1	1	1			
Pleurotropis fasciatis Gir.[2]	1	16	16	15	341	22+
Cirrospilus pictus (Nees)				2	30	15
Dibrachys cavus (Walk.)[3]	125	1,875	15	405	6,049	15
Eurytoma sp.	1	1	1			
Eupteromalus sp.[4] (gregarious)	12	210	17+	18	242	13
Eupteromalus sp.[4] (solitary)	4	4	1	29	29	1
Total killed by parasites	240			725		
Adults issued	1,528			3,583		
Dead from unknown causes	307			757		
Total cocoons collected	2,075			5,065		

[1] Determinations made by C. F. W. Muesebeck, A. B. Gahan, and R. A. Cushman, of the Bureau of Entomology and Plant Quarantine, Division of Insect Identification.

[2] 1 cocoon produced 16 *Pleurotropis* and 4 *Dibrachys*.

[3] 1 cocoon produced 3 *Dibrachys* and 1 *Eupteromalus*.

[4] A. B. Gahan, of the Bureau of Entomology and Plant Quarantine, Division of Insect Identification, states that this species of *Eupteromalus* is "very close" to *E. nidulans* Foerst., and that the large solitary specimens are probably large specimens of the same species.

De Fluiter (*4*) reared *Dibrachys? boucheanus* Ratz. (=*cavus* Walk.?), *Eurytoma appendigaster* Boh., *Habrocytus emerus* (Ratz.), and *Mesochorus marginatus* Thoms. from *Rogas* cocoons in Holland. *D. cavus* is the only one of these species that was also present at Budapest.

Rogas spends about 12 days in the cocoon, but all the hyperparasites except the two species of *Mesochorus* attack this stage. *Mesochorus tuberculiger* Thoms. attacks the tiny hibernating *Rogas* inside the *Stilpnotia salicis* larva. *Mesochorus* females pay practically no attention to unparasitized *S. salicis*, but upon encountering a parasitized larva the hyperparasite strokes it rapidly with its antennae and jabs it repeatedly with its ovipositor. Upon dissection the attacked *Rogas* larva is found to contain a tiny gourd-shaped *Mesochorus* egg.

FACTORS LIMITING THE EFFECTIVENESS OF THIS PARASITE

The only obvious factor limiting the effectiveness of *Rogas unicolor* as a parasite of *Stilpnotia salicis* is the hyperparasitism. There are numerous parasites, however, that are apparently just as severely attacked by hyperparasites, which prove to be far more effective as control factors of their respective hosts. Although *S. salicis* has been reared at many points in Europe, *R. unicolor* has been recorded in appreciable numbers only at Budapest, Hungary, and at Wageningen, Holland. It seems that there must be some unknown factors, possibly climatic, that keep it from becoming a more important parasite of this host in Europe.

SUMMARY

Rogas unicolor (Wesm.) is a braconid parasite of minor importance on *Stilpnotia salicis* L. in Europe. In 1933 and 1934 about 7,000 cocoons were collected at Budapest, Hungary, and sent to the United States. Adults were colonized in New England and in Washington, but the species has not yet been definitely recovered.

The various stages of the parasite are described in detail.

Rogas unicolor has one generation a year. The winter is passed as a first-instar larva within the hibernating host larva. Development proceeds slowly in the spring, and the parasite becomes full grown when the host larva is in the penultimate instar. The cocoon is formed inside the skin of the dead host larva. About 12 days is spent in the cocoon, and adults issue the last of June. Parthenogenetic reproduction results in female progeny, although an occasional male appears. A maximum of 322 eggs was obtained from a single female.

Host larvae that are parasitized by *Rogas unicolor* are considerably retarded in growth. Just before the parasite becomes full grown, the host larva spins a dense white web, within which the parasite cocoon is formed.

Rogas unicolor is attacked by a number of hyperparasites. Fourteen species were reared from *Rogas* cocoons collected in the field at Budapest, Hungary. The most important hyperparasite was *Dibrachys cavus* (Walk.), which has been noted by Fiske as an important parasite of "*Rhogas intermedias* (Cresson)" in New England. Hyperparasitism is the most apparent factor limiting the effectiveness of this parasite, although climate or other, unknown factors may actually be more important.

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TRANSMISSION OF SUGARCANE MOSAIC BY THE GREEN BUG (TOXOPTERA GRAMINUM ROND.)[1]

By J. W. INGRAM, *entomologist, Division of Cereal and Forage Insect Investigations, Bureau of Entomology and Plant Quarantine,* and E. M. SUMMERS, *assistant pathologist, Division of Sugar Plant Investigations, Bureau of Plant Industry, United States Department of Agriculture*[2]

INTRODUCTION

In 1920 Brandes[3] reported experiments showing that sugarcane mosaic was transferred by the corn leaf aphid (*Aphis maidis* Fitch). This remained the only proved vector until 1936, when the present authors[4] reported a series of successful transfers with the rusty plum aphid (*Hysteroneura setariae* (Thos.)) and stated that the green bug (*Toxoptera graminum* Rond.) had been observed to transfer mosaic in two cases. Additional experiments, conducted during 1936, gave further proof that the green bug is a vector, and the results of these experiments are reported herein.

DISTRIBUTION AND HABITS OF THE GREEN BUG

Toxoptera graminum is a species of world-wide distribution, having been reported from India, Africa, Australia, and Europe, as well as from North America. It has been found in nearly all parts of the United States. In the Mississippi River Basin it causes heavy damage to small-grain crops, principally wheat and oats. This aphid has not been reported as feeding on sugarcane elsewhere than in Louisiana and not until recently in that State.

In the sugarcane section of Louisiana this aphid has been observed chiefly on sugarcane, Johnson grass (*Sorghum halepense*), crabgrass (*Digitaria sanguinalis*), *Panicum dichotomiflorum*, and *P. reptans*. In many fields it has been found in numbers about equal to those of *Aphis maidis* on sugarcane, but generally it is not so abundant. The green bug occurs in greatest abundance on crabgrass. Large numbers have also been seen on Johnson grass, which is sometimes found growing in sugarcane fields but more frequently on ditchbanks and headlands surrounding the fields. None of these aphids were ever found on oats growing alongside a sugarcane field in which there was a heavy infestation on crabgrass.

Both winged and wingless forms are commonly found in the whorls of sugarcane plants, where they have undoubtedly been mistaken for *Aphis maidis* in the past. *Toxoptera graminum* also feeds on the other parts of the plant. It is sometimes observed feeding at the tips of the leaves on the under side. Since the green color of this aphid is more nearly like that of sugarcane than is the color of any of the other aphids occurring on sugarcane, it is the most difficult species to find.

[1] Received for publication December 15, 1937; issued May 1938.
[2] The authors gratefully acknowledge helpful suggestions from P. N. Annand, of the Bureau of Entomology and Plant Quarantine, and R. D. Rands, of the Bureau of Plant Industry.
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ATTEMPTED TRANSFERS FROM MOSAIC SUGARCANE TO HEALTHY SUGARCANE

Parallel experiments were conducted in which *Toxoptera graminum* was compared with *Aphis maidis*, which still appears to be the most efficient vector of sugarcane mosaic. Since Co. 281 is one of the generally planted varieties suffering serious injury from mosaic in Louisiana, it was used in these experiments.

Single bud cuttings of mosaic cane, to be used as a source of inoculum, and pedigreed healthy cane were planted in 4-inch pots of sterilized soil, sprouted in incubators, and held in an insect-proof greenhouse until ready for use. When they were from 3 to 6 inches high, the mosaic plants were transferred to cloth and glass insect-proof cages in the insectary. These cages were either 15 by 28 by 49 or 16 by 30 by 49 inches high. Aphids were collected from nonmosaic host plants and placed on the mosaic plants at the rate of about 50 per plant. Separate cages were maintained for each species of aphid. Thirty viruliferous aphids were transferred to each healthy cane plant 24 to 26 hours later, and these plants were then placed in insectary cages of the type just described. In from 5 to 7 days these plants were fumigated. Check plants were given the same treatment except for the absence of aphids from the cages.

TABLE 1.—*Summary of mosaic-transmission experiments with Toxoptera graminum and Aphis maidis in 1936*

Date of transfer of insects	Species of aphid used	Healthy plants used	Healthy plants developing mosaic	Period of exposure before mosaic symptoms appeared
		Number	*Number*	*Days*
Apr. 8	*T. graminum*	32	4	15 (2), 19 (2).[1]
	A. maidis	23	4	14, 17 (2), 26.
	Check	7	0	
Apr. 15	*T. graminum*	2	0	
Apr. 27	*T. graminum*	10	1	18.
	A. maidis	6	2	12 and 14.
	Check	6	0	
Apr. 29	*T. graminum*	23	2	10 and 12.
	A. maidis	21	8	12, 14 (2), 16, 19, 21, 26, 54.
	Check	6	0	
May 1	*T. graminum*	28	4	14 (3) and 27.
	A. maidis	32	20	13 (3), 14 (7), 15 (4), 18, 20 (3), 22, 25.
	Check	10	0	
May 4	*T. graminum*	15	4	11, 16 (2), 21.
	A. maidis	17	3	16, 46 (2).
	Check	7	0	
May 6	*T. graminum*	28	2	19 and 45.
	A. maidis	11	1	45.
	Check	5	0	
May 8	*T. graminum*	28	3	17 and 33 (2).
	Check	15	0	
May 29	*T. graminum*	1	0	
	A. maidis	8	1	18.
	Check	7	0	
June 2	*T. graminum*	5	1	14.
	A. maidis	6	1	14.
	Check	7	0	

[1] Numbers in parentheses indicate the number of plants that developed mosaic after the number of days that are given immediately preceding the parentheses.

Immediately after fumigation all plants with the surrounding soil were transferred to flats in the greenhouse, where they remained located on ant-proof benches until discarded. No aphids were found in the greenhouse, although frequent examinations were made. Plants

were examined at daily or, in some cases, 2-day intervals, for the first appearance of mosaic symptoms. The plants were given a liberal application of nitrogen fertilizer and kept under as favorable growing conditions as possible. All plants used in a given experiment were derived from the same source, were of the same age, and were grown under similar conditions.

The results of these experiments are shown in table 1. It may be noted that there were 21 mosaic transfers to originally healthy cane in the 172 plants exposed to viruliferous *Toxoptera graminum* as compared with 40 out of 124 healthy plants similarly infested with *Aphis maidis* in parallel experiments. No mosaic appeared in the 70 check plants or in any plants of similar origin growing in the greenhouse in other experiments.

ATTEMPTED TRANSFERS FROM MOSAIC CRABGRASS TO HEALTHY SUGARCANE

Crabgrass is the most abundant of the grasses that show symptoms of mosaic in sugarcane fields. Since *Toxoptera graminum* has often been found in large numbers on this grass, a few experiments were conducted to determine whether this aphid is capable of transferring mosaic from crabgrass to sugarcane. Experiments were conducted in cages as in previous tests.

In an experiment begun on July 29, 30 individuals of *Toxoptera graminum* from mosaic crabgrass were transferred to each of 36 healthy plants of P. O. J. 234 sugarcane. Seven days later the infested plants were fumigated. No live aphids were observed on the plants prior to fumigation; they had died soon after being transferred as a result of parasitization and high temperatures. There was no transfer of mosaic.

On August 10, 30 *Toxoptera graminum* aphids from crabgrass were transferred to each of 14 healthy plants of P. O. J. 234 sugarcane. Although the aphids died soon after the transfer, one plant showed mosaic 18 days after exposure to aphids. None of the 14 parallel check plants, given similar treatment except for exposure to aphids, developed mosaic.

On July 31, 30 individuals of *Toxoptera graminum* were transferred from mosaic crabgrass to each of 4 healthy crabgrass plants. The plants were fumigated 5 days later. None of these plants developed mosaic.

DISCUSSION AND CONCLUSIONS

In experiments conducted in 1936 *Toxoptera graminum* transferred mosaic from diseased to healthy sugarcane plants in 21 cases. These transfers, together with 2 transfers obtained in 1935 and previously reported and 1 from mosaic crabgrass, make a total of 24 transfers of mosaic to healthy sugarcane by this aphid. This establishes *T. graminum* as a vector of sugarcane mosaic.

From results reported here it is calculated that 12 percent of the healthy plants developed mosaic when infested with viruliferous *Toxoptera graminum*, and 32 percent developed mosaic when infested with *Aphis maidis* in parallel experiments. On the basis of these limited observations and of insect-population counts in and around sugarcane fields, it appears that *T. graminum* is of less importance than *A. maidis* as a vector of sugarcane mosaic.

Proof of mosaic transfer by the green bug is important, however, since it gives a third known insect vector of this disease in Louisiana. The time required for the appearance of mosaic symptoms, as seen from data given in the table, was about the same with *Aphis maidis* and *Toxoptera graminum*. Although the data on this point are limited and therefore not conclusive, such similarity would be expected, since both species ordinarily feed in the same place, that is, in the central whorl of the plant.

The discovery of this additional vector further complicates the problem of decreasing mosaic spread by means of vector control. Even though the green bug may not usually be a major agent in the spread of mosaic, it will have to be considered in any control program, because its increase in numbers under certain weather, host-plant, or other conditions may cause it to become an important vector of mosaic disease in sugarcane.

SUMMARY

Data reported in 1920 showed that the corn leaf aphid (*Aphis maidis* Fitch) was a vector of sugarcane mosaic. In 1936 the rusty plum aphid (*Hysteroneura setariae* (Thos.)) was shown to be a vector of the same disease, and two transfers of mosaic by the green bug (*Toxoptera graminum* Rond.) were reported.

The green bug is fairly generally distributed throughout the world. It is found in nearly all parts of the United States, and causes heavy injury to small grains in the Mississippi River Valley. These aphids feed upon various parts of sugarcane plants that are above ground and on a number of other grasses commonly found in and around sugarcane fields.

In a series of experiments conducted during 1936, 30 *Toxoptera graminum* from infected sugarcane plants were transferred to each of 172 healthy plants, and 21 of these developed mosaic. In a parallel experiment 40 of 124 healthy plants exposed to viruliferous *Aphis maidis* developed mosaic symptoms. One transfer of the mosaic resulted from the green bug from infected crabgrass being colonized on healthy sugarcane.

The data indicate that the green bug is not, in general, so important in field transfer of sugarcane mosaic as either of the other two vectors, but that it may be the most important transfer agent in certain fields.

A MOSAIC DISEASE OF CHINESE CABBAGE [1]

By C. M. TOMPKINS, *assistant plant pathologist*, and H. REX THOMAS, formerly *graduate assistant in plant pathology, California Agricultural Experiment Station* [2]

INTRODUCTION

In October 1934, a mosaic disease of Chinese cabbage (*Brassica pe-tsai* Bailey) was observed in the northern part of the San Joaquin Valley and later in the Salinas Valley of California. Although natural infection in this and subsequent years varied from 60 to 100 percent, negligible losses resulted and the heads usually attained marketable size. Preliminary mechanical inoculations to Chinese cabbage and certain other cruciferous test plants in the greenhouse were successful and indicated that this virus was apparently different from the cauliflower mosaic, annual stock mosaic, and cabbage black ring viruses previously reported from this State (*11*, *12*, *13*).[3] In addition to presenting the results of studies of the disease, this paper briefly compares the Chinese cabbage mosaic virus with certain other crucifer viruses.

REVIEW OF LITERATURE

Apparently the first published record of a mosaic disease on Chinese cabbage was contributed by Schultz (*9*) in 1921. He noted that infected plants were dwarfed, with mottled, ruffled, and distorted leaves. Mustard (*B. japonica* Sieb.) and turnip (*B. rapa* L.) in nearby areas were similarly affected. By means of juice inoculations, he obtained intertransmission between these hosts after 20 to 30 days; symptoms developed in 12 to 30 days when infective green peach aphids were transferred to healthy plants. Simultaneously, Gardner and Kendrick (*5*) described a mosaic disease on turnip, and it seems highly probable that their virus was identical with that studied by Schultz (*9*).

In 1924 Kunkel (*7*) found intracellular bodies in mosaic-diseased leaves of Chinese cabbage plants which had earlier been collected in Honolulu gardens. In a later communication,[4] he stated that the disease "caused some distortion of leaves and stunting of the plants but was not particularly severe."

Under greenhouse temperatures ranging from 70° to 80° F., Clayton (*1*) determined that Chinese cabbage could be readily infected by juice inoculations with the rutabaga mosaic virus. The plants showed inconspicuous mottling, stunting, and streak symptoms.

A mosaic disease of Chinese cabbage, turnip, and mustard was observed in Fukuoka, Japan, in 1927 by Takimoto (*10*). Crop losses frequently approached 30 percent. Characteristic symptoms consisted of dwarfed, mottled, and deformed leaves. The virus was shown to be transmitted by means of aphids (species not given).

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[2] The writers are indebted to Profs. Ralph E. Smith and M. W. Gardner for advice and assistance and to Prof. B. A. Madson and W. W. Mackie for providing greenhouse space and facilities. Valuable assistance in the greenhouse work was rendered by employees of the Federal Works Progress Administration.

[3] Reference is made by number (italic) to Literature Cited, p. 551.

[4] Letter dated December 20, 1934, from L. O. Kunkel, Department of Animal and Plant Pathology, The Rockefeller Institute for Medical Research, Princeton, N. J.

Cauliflower (*Brassica oleracea* L. var. *botrytis* L.) was infected by means of juice inoculations, but not cabbage (*B. oleracea* L. var. *capitata* L.) or radish (*Raphanus sativus* L.). Since Gardner and Kendrick (*5*) were also unable to obtain infection of radish with the turnip mosaic virus, it is possible that the Japanese virus may be closely related to strains of the turnip virus found in the United States.

Further references to the occurrence of a mosaic disease of Chinese cabbage in Japan were published in 1932 and 1933 by Fukushi (*4*) and Hino (*6*), respectively.

Accordingly to Weber (*14*), a mosaic disease of Chinese cabbage is of minor importance in Florida, causing negligible losses. The identity of the virus was not mentioned.

In Chinese gardens in Manila, P. I., 30 to 50 percent infection of Chinese cabbage plants was not uncommon, according to Fajardo (*3*).

SYMPTOMS OF THE DISEASE

As observed under greenhouse conditions, the first symptoms of the mosaic disease of Chinese cabbage consist of a pronounced systemic clearing of the veins, which generally commences at or near the base of the leaf and gradually spreads over the entire leaf (fig. 1, *A*, *B*). After 3 to 4 weeks the early symptoms on the young, inner leaves commence to change gradually into a very conspicuous, coarse type of mottling (fig. 1, *C*), in marked contrast to healthy leaves (fig. 1, *D*). The irregular, light- and dark-green areas between the veins, which give rise to the mottled appearance, cause little or no distortion of the leaf surfaces. Raised, dark-green islands on the leaves, which characterize the mosaic diseases of certain plants, were not observed on any of several thousand artificially-infected plants used during the coarse of these studies. Usually the older, outer leaves of infected plants continue to show clearing of the veins without other change, and this condition persists until they turn yellow and die. The disease causes a mild stunting of the entire plant, which may not be apparent, however, if infection occurs late.

MATERIALS AND METHODS

The Chinese cabbage mosaic virus which was used in the experimental studies originated from a mature, systemically infected Chinese cabbage plant grown in the Salinas Valley. After the virus had been established on young Chinese cabbage plants in the greenhouse by mechanical inoculation, successive transfers were made to healthy seedlings at frequent intervals in order to provide a constant source of fresh inoculum.

Temperatures in the greenhouse in which these tests were conducted ranged from 13° to 19° C. The methods followed were essentially those described in a recent paper (*12*). Mechanical inoculations were made by dusting the leaves with powdered carborundum (600 mesh) and rubbing lightly with a piece of cotton soaked with the juice from a diseased plant.

Chinese pe-tsai cabbage seedlings, four to six leaves, served as test plants for both property studies and recovery of the virus. The experimental host range of the virus was determined by means of mechanical inoculations in the greenhouse.

TRANSMISSION

Preliminary inoculations of Chinese cabbage by rubbing the leaves with expressed juice gave approximately 50-percent infection. Further tests resulted in 100 percent infection when carborundum (*8*) supplemented this method. The use of carborundum tended slightly to decrease the incubation period, which was determined as ranging from 13 to 22 days.

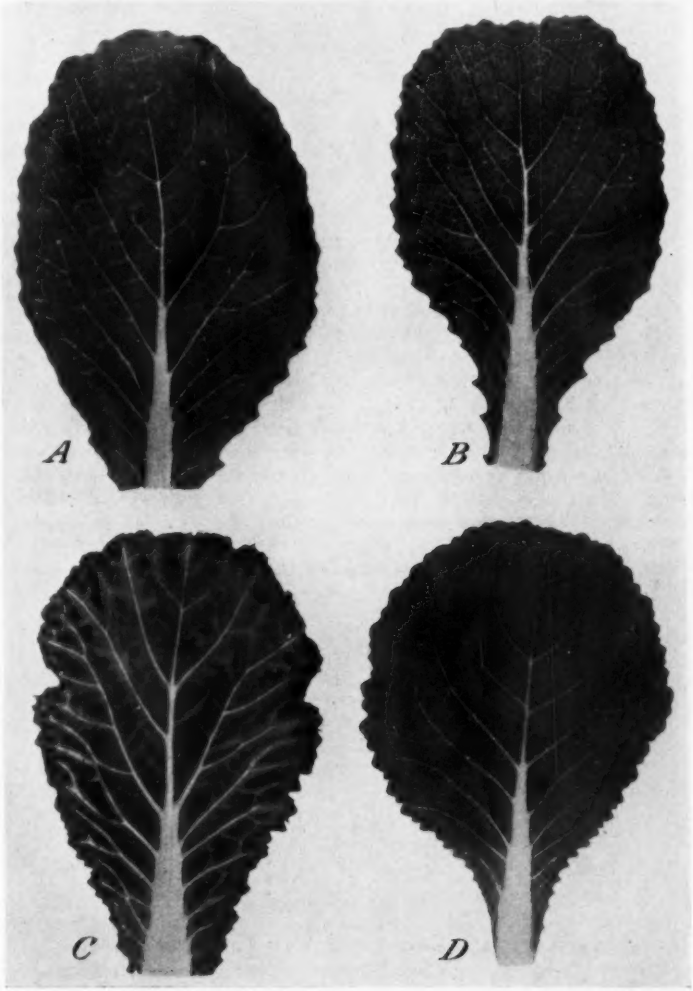

FIGURE 1.—Symptoms produced by the Chinese cabbage mosaic virus on Chinese cabbage leaves by mechanical inoculation in the greenhouse at 13° to 19° C.: *A*, Early stage of vein clearing; *B*, advanced stage of vein clearing; *C*, late stage of infection showing coarse type of mottling; *D*, noninoculated control.

Studies on insect transmission of this disease involved the use of the cabbage aphid (*Brevicoryne brassicae* (L.)) and the green peach aphid (*Myzus persicae* (Sulzer)), both of which, according to Essig (*2*), breed naturally on cabbage, cauliflower, and other cultivated and wild cruciferous plants. Schultz (*9*) transmitted the crucifer virus with which he worked to Chinese cabbage by means of the green peach aphid only.

Noninfective cabbage and green peach aphids [5] were colonized on healthy cauliflower and sugar-beet (*Beta vulgaris* L.) plants under cloth cages in the greenhouse. When the population had increased sufficiently, detached leaves with aphids were placed on diseased Chinese cabbage plants under cages to permit of natural migration. After the aphids had fed for 24 to 48 hours, transfers in lots of approximately 20 infective aphids each were made to individual, healthy Chinese cabbage seedlings, as outlined above. Chinese cabbage seedlings infested with noninfective aphids served as controls, plus a number of noninoculated plants which were free from aphids.

In all, 60 Chinese cabbage seedlings were tested with infective cabbage aphids, of which 56 showed typical symptoms of the disease within 14 to 23 days. Of 20 plants tested with infective green peach aphids, 17 became diseased after 17 to 23 days. The virus was recovered from all infected plants by the carborundum method. All controls remained healthy.

Tests for seed transmission were limited to seed obtained from 19 diseased Chinese cabbage plants which had previously been inoculated by mechanical means in the greenhouse. Seeds were planted in a flat of autoclaved soil. After 21 days, 477 plants were examined, all of which were healthy, indicating that the virus is probably not carried in the seed. Schultz (*9*) planted mustard seed derived from mosaic mustard plants and concluded that there was no evidence of seed transmission.

SUSCEPTIBILITY OF CHINESE CABBAGE VARIETIES TO ARTIFICIAL INFECTION

Young seedlings of the following varieties of Chinese cabbage were tested by mechanical inoculation in the greenhouse to determine their susceptibility to infection: Chinese pe-tsai (several strains), Chinese Chicken, Chinese Chihli, and three Japanese selections of pe-tsai (Hootooren, Nagasaki Hakusai, and Tsuzita Hakusai). A suitable number of plants of each variety was reserved for controls. All varieties proved highly susceptible and the incubation period for each was within the limits of 13 to 22 days.

EXPERIMENTAL HOST RANGE

Inoculation tests indicate that the virus becomes systemic only in cruciferous plants, though local lesions were produced on two species of the Solanaceae (table 1). A brief description of the symptoms produced on each host is given in table 1, and characteristic symptoms on different hosts are shown in figures 2, *A*, *B*, *C*, *D*, and figure 5, *A*.

[5] Aphids identified by E. O. Essig, as noninfective cabbage and green peach aphids, were kindly supplied by H. H. Severin and J. H. Freitag, Division of Entomology and Parasitology.

TABLE 1.—*Plants susceptible to the Chinese cabbage mosaic virus, as indicated by mechanical inoculation of greenhouse-grown seedlings, and symptoms characteristic of infection*

Family	Species and common name	Symptoms produced
Cruciferae	*Brassica oleracea* L. var. *botrytis* L. (cauliflower—variety February).	Systemic. Numerous chlorotic rings, best seen by transmitted light.
	B. oleracea L. var. *capitata* L. (cabbage—var. Winter Colma).	Systemic. Very coarse, yellowish vein banding.
	B. napus L. (rape)	Systemic. Coarse mottling.
	B. rapa L. (turnip—var. Purple Top White Globe).	Systemic. Fine type of vein clearing and mottling.
	Matthiola bicornis DC. (evening scented stock).	Systemic. Coarse mottling, with marked chlorosis and rosetting of the terminal growth.
	M. incana R. Br. var. *annua* Voss (annual stock—var. Fiery Blood Red).	Systemic. Fine type of mottling and flower breaking.
	Lunaria annua L. (honesty)	Systemic. Vein clearing.
	Malcomia maritima R. Br. (Virginian stock).	Do.
	Raphanus sativus L. (radish—var. White Icicle).	Systemic. Vein clearing and mottling.
	Capsella bursa-pastoris (L.) Medic. (shepherds-purse).	Systemic. Mottling.
	B. adpressa Boiss.	Systemic. Chlorotic ring pattern, producing somewhat of a mottled effect.
	B. arrensis (L.) Ktze. (charlock)	Systemic. Vein clearing.
Solanaceae	*Nicotiana glutinosa* L.	Not systemic. Symptoms on inoculated leaves only, consisting of numerous chlorotic rings.
	N. tabacum L. (tobacco—var. Turkish)	Not systemic. Symptoms on inoculated leaves only, consisting of numerous chlorotic rings with dark-green edges.

Unsuccessful attempts were made to transmit the virus to 54 species of plants representing 46 genera in 27 families, as follows: Corn (*Zea mays* L.) var. Golden Bantam; oat (*Avena sativa* L.); rhubarb (*Rheum rhaponticum* L.); lambsquarters or white pigweed (*Chenopodium album* L.); sowbane or nettle leaf goosefoot (*Chenopodium murale* L.); spinach (*Spinacia oleracea* L.) var. Bloomsdale; sweet-william (*Dianthus barbatus* L.); babysbreath (*Gypsophila paniculata* L.); rocket larkspur (*Delphinium ajacis* L.); hyprid delphinium (*D. cultorum* Voss) Blackmore & Langdon strain; Iceland poppy (*Papaver nudicaule* L.); oriental poppy (*P. orientale* L.); *Brassica integrifolia* O. E. Schulz var. *chevalieri* R. Porteres; leaf or Chinese mustard (*B. juncea* Coss); white mustard (*B. alba* (L.) Boiss.); mignonette (*Reseda odorata* L.); *Geum chiloense* Balb.; garden pea (*Pisum sativum* L.) var. Alderman; broadbean (*Vicia faba* L.); storksbill (*Pelargonium zonale* Willd.); garden nasturtium (*Tropaeolum majus* L.); castor-bean (*Ricinus communis* L.); pansy (*Viola tricolor* L.); fibrous-rooted begonia (*Begonia semperflorens* Link and Otto); *Clarkia elegans* Dougl.; *Godetia grandiflora* Lindl.; celery (*Apium graveolens* L.) var. Golden Self Blanching; forget-me-not (*Myosotis alpestris* Schmidt); common heliotrope (*Heliotropium peruvianum* L.); garden verbena (*Verbena hybrida* Voss); flowering sage (*Salvia farinacea* Benth.); *Solanum aviculare* Forst.; potato (*S. tuberosum* L.) var. Katahdin; tomato (*Lycospersicum esculentum* Mill. var. *vulgare* Bailey) var. Early Santa Clara Canner; currant tomato (*L. pimpinellifolium* Dunal); *Nicotiana langsdorffii* Weinm.; jimsonweed (*Datura stramonium* L.); petunia (*Petunia hybrida* Hort.); snapdragon (*Antirrhinum majus* L.); beardtongue (*Pentstemon barbatus* Nutt.); mourning bride (*Scabiosa atropurpurea* L.); cucumber (*Cucumis sativus* L.); canterbury-bells (*Campanula medium* L.); lobelia (*Lobelia hybrida* Hort.); dandelion (*Taraxacum officinale* Weber); head lettuce (*Lactuca sativa* L. var.

capitata Hort.) var. New York and Tom Thumb; annual marguerite (*Chrysanthemum coronarium* L.); China-aster (*Callistephus chinensis* Nees) var. Giant Branching White, wilt-resistant; English daisy

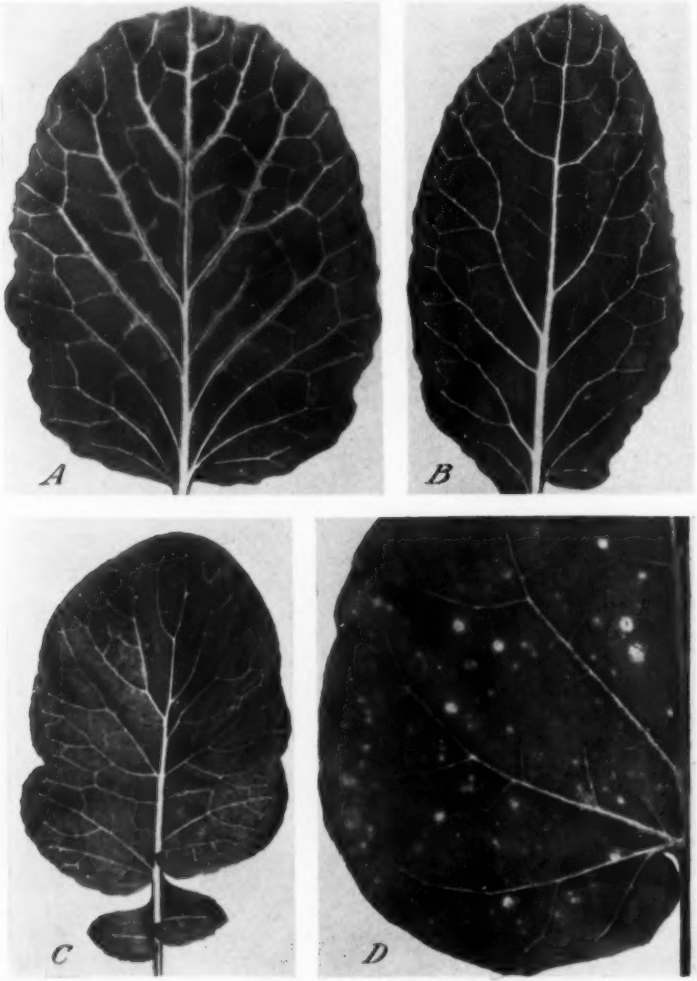

FIGURE 2.—Symptoms produced by the Chinese cabbage mosaic virus on leaves of certain plants by mechanical inoculation in the greenhouse at 13° to 19° C.: *A*, Winter Colma cabbage; *B*, February cauliflower; *C*, White Icicle radish; *D*, *Nicotiana glutinosa*.

(*Bellis perennis* L.); African marigold (*Tagetes erecta* L.); French marigold (*T. patula* L.); *Gaillardia pulchella* Foug. var. *picta* Gray; winter Cape-marigold (*Dimorphotheca aurantiaca* DC.); and cineraria (*Senecio cruentus* DC.).

PROPERTIES OF THE VIRUS

In table 2, the results of property studies of the Chinese cabbage mosaic virus are given. All virus samples, consisting of 2 cc of undiluted juice from diseased plants, were contained in small, stoppered, thin-walled test tubes. For longevity tests, virus samples were stored at a constant temperature of 22° C. The virus retained its infectivity for 72 hours but was inactivated after 96 hours. The inactivation temperature lies between 73° and 75° for 10-minute exposure, which closely approaches that established for the cauliflower mosaic virus (*12*). Infection was obtained when the virus was diluted to 1 to 5,000, but higher dilutions inactivated it. In each of the three tests, 25 noninoculated plants served as controls, and they continued healthy throughout.

TABLE 2.—*Longevity in vitro, inactivation temperature, and tolerance to dilution of the Chinese cabbage mosaic virus*

[25 plants inoculated in 5 trials in each case]

LONGEVITY IN VITRO, 22° C.							
Aged (hours)	Trials	Plants inoculated	Plants infected	Aged (hours)	Trials	Plants inoculated	Plants infected
	Number	*Number*	*Number*		*Number*	*Number*	*Number*
0	5	25	21	72	5	25	3
4	5	25	16	96	5	25	0
8	5	25	8	120	5	25	0
24	5	25	14	144	5	25	0
48	5	25	8	168	5	25	0
INACTIVATION TEMPERATURE (10 MINUTES)							
Temperature (°C.)	Trials	Plants inoculated	Plants infected	Temperature (°C.)	Trials	Plants inoculated	Plants infected
	Number	*Number*	*Number*		*Number*	*Number*	*Number*
50	5	25	25	73	5	25	8
55	5	25	25	75	5	25	0
60	5	25	25	80	5	25	0
65	5	25	16	(1)	5	25	25
70	5	25	14				
TOLERANCE TO DILUTION							
Dilution	Trials	Plants inoculated	Plants infected	Dilution	Trials	Plants inoculated	Plants infected
	Number	*Number*	*Number*		*Number*	*Number*	*Number*
0	5	25	24	1:2000	5	25	2
1:10	5	25	22	1:3000	5	25	2
1:25	5	25	15	1:4000	5	25	2
1:100	5	25	12	1:5000	5	25	1
1:200	5	25	6	1:6000	5	25	0
1:500	5	25	4	1:7000	5	25	0
1:1000	5	25	2	1:8000	5	25	0

[1] Not treated.

DESCRIPTION OF THE CHINESE CABBAGE MOSAIC VIRUS

Transmitted in greenhouse tests by means of *Brevicoryne brassicae* (L.) and *Myzus persicae* (Sulzer). Transmissible by mechanical inoculation with expressed juice, with or without powdered carborundum. Incubation period 13 to 22 days. Resistance to aging in vitro between 3 and 4 days. Inactivation temperature between 73° and 75° C. for 10-minute exposure. Tolerance to dilution approximately 1 to 5,000. Chinese cabbage (*Brassica pe-tsai* Bailey) and certain other vegetable and ornamental crucifers susceptible. On Chinese cabbage, symptoms consist of slight stunting of the plant; vein clearing followed by mottling, with but slight distortion of leaves and no necrotic lesions or midrib curvature. Local lesions produced on *Nicotiana tabacum* L. and *N. glutinosa* L. but not on *N. langsdorffii* Weinm.

COMPARISON OF THE CHINESE CABBAGE MOSAIC VIRUS WITH CERTAIN OTHER CRUCIFER VIRUSES

In the course of these studies, the Chinese cabbage mosaic virus was compared with the cauliflower mosaic and turnip mosaic (from New York) viruses (*12*) for symptom expression by means of parallel

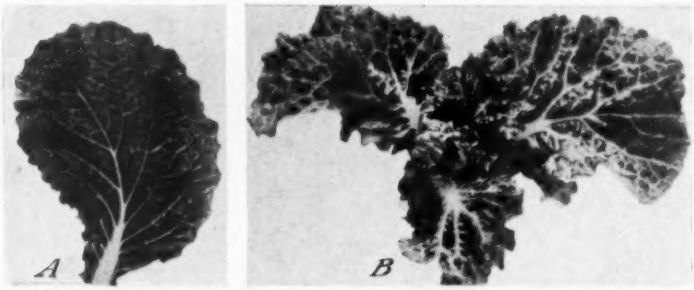

FIGURE 3.—Symptoms produced by the cauliflower mosaic virus on leaves of Chinese cabbage by mechanical inoculation in the greenhouse at 13° to 19° C.: *A*, Leaf distortion and curvature of the midrib are characteristic effects; *B*, stunting of the plant.

mechanical inoculations made simultaneously to Chinese cabbage, Winter Colma cabbage, February cauliflower, and Purple Top White Globe turnip seedlings in the greenhouse.

On Chinese cabbage, the symptoms produced by the Chinese cabbage mosaic virus have been described. The cauliflower mosaic virus causes systemic infection after 20 days, the symptoms consisting of a conspicuous clearing of the veins which in the early stages cannot be distinguished from the vein-clearing symptom characteristic of the early effects of the Chinese cabbage mosaic virus. Within a few days, however, vein clearing is supplemented by curvature of the midrib, with considerable distortion and ruffling of the leaf (fig. 3, *A*). Vein clearing persists as the leaves become older, but no instances of mottling were observed. The entire plant is severely stunted (fig. 3, *B*). These symptoms are specific to the cauliflower mosaic virus and as such offer a ready means of identification.

Systemic infection of Chinese cabbage plants with the turnip mosaic virus occurred 21 days after inoculation. Symptoms were most pronounced on the outer or older leaves of the plant and consisted of a coarse, yellowish type of vein banding (fig. 4). Since Schultz (*9*) observed mosaic mottling on infected Chinese cabbage plants in both the field and greenhouse, rather than vein clearing as observed by

the writers, the existence of strains of the turnip mosaic virus is indicated.

On Winter Colma cabbage, the symptoms produced by the Chinese cabbage mosaic virus consisted of a very coarse, yellowish, vein banding (fig. 2, *A*). The cauliflower mosaic virus caused vein clearing only (*12*, *fig. 3*, *C*), while the turnip mosaic virus from New York induced light-green to yellow lesions whose edges finally became necrotic (*12*, *fig. 5*, *A*). Cabbage proved to be an excellent differential host.

On February cauliflower, the Chinese cabbage mosaic virus causes systemic infection, consisting of scattered chlorotic rings with dark-green centers. These rings, with an average diameter of one-eighth of an inch, do not become necrotic with age and, being barely discernible, are best viewed by means of transmitted light (fig. 2, *B*). The symptoms produced by the cauliflower mosaic virus on this host consist of vein clearing, followed by vein banding, mottling, and necrotic lesions (*12*, *fig. 1*, *A*, *B*, *C*). Although the turnip mosaic virus also produced a chlorotic green to yellow ring pattern on cauliflower (*12*, *fig. 5*, *B*), the lesions cannot be confused with those caused by the Chinese cabbage mosaic virus. The latter do not turn yellow and are much more diffuse (fig. 2, *B*).

FIGURE 4.—Symptoms produced by a turnip mosaic virus from New York on leaves of Chinese cabbage by mechanical inoculation in the greenhouse at 13° to 19° C., showing coarse, yellow vein banding.

Purple Top White Globe turnip seedlings proved to be the best differential host for ready separation of the three viruses on the basis of symptoms. The Chinese cabbage mosaic virus caused systemic vein clearing after 20 days, followed by a very fine type of mottling (fig. 5, *A*). The cauliflower mosaic virus caused vein clearing but no mottling (*12*). A very coarse, contrastive type of mottling, accompanied by leaf ruffling and raised areas or dark-green blisters (fig. 5, *B*) characterized infection by the turnip mosaic virus.

A comparison of the three viruses as to properties cannot be made since no data are yet available for the turnip mosaic virus from New York. If the Chinese cabbage mosaic virus is compared with the

cauliflower mosaic virus (*12*), it will be observed that the longevity in vitro of the former is 3 days as against 15 days for the latter; the inactivation temperatures tend to approach each other. A dilution tolerance of 1 to 5,000 was obtained for the Chinese cabbage mosaic virus as compared to 1 to 2,000 for the cauliflower mosaic virus.

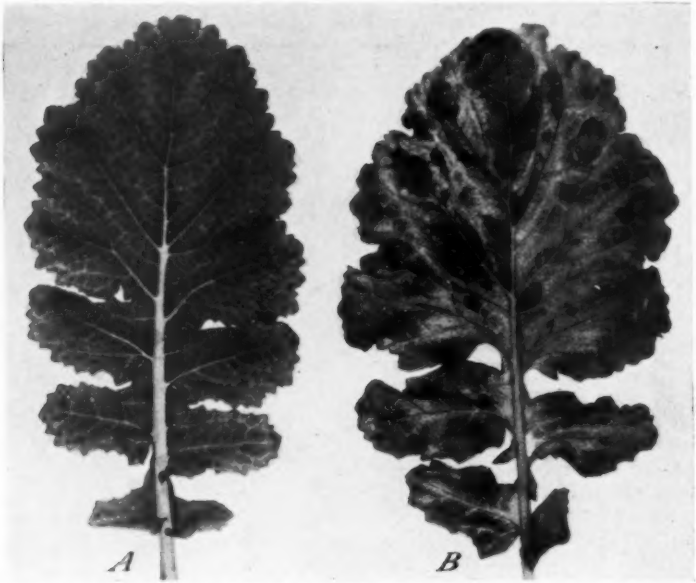

FIGURE 5.—Symptoms produced on leaves of Purple Top White Globe turnip by mechanical inoculation in the greenhouse at 13° to 19° C.: *A*, By the Chinese cabbage mosaic virus, which induces vein clearing and mottling; *B*, by a turnip virus from New York which causes a coarse, bold mottle.

SUMMARY

A mosaic disease of Chinese cabbage (*Brassica pe-tsai*), prevalent in central California during the fall and winter months, is described.

The symptoms of the disease consist of a systemic clearing of the veins, followed by general mottling, with little or no distortion of the leaves.

In the greenhouse, transmission of the virus was obtained by mechanical inoculation with carborundum and also by means of the cabbage aphid (*Brevicoryne brassicae*) and the green peach aphid (*Myzus persicae*). Seed transmission tests gave negative results.

The virus was infectious after storage for 3 days at 22° C.; after a 10-minute treatment in a water bath at 73°; and after being diluted 1 to 5,000.

Local lesions were obtained on *Nicotiana glutinosa* and *N. tabacum*, but aside from these the host range was confined to the family Cruciferae.

Considered as to symptoms, the Chinese cabbage mosaic, cauliflower mosaic, and turnip mosaic viruses can easily be differentiated on Chinese cabbage, Winter Colma cabbage, and Purple Top White Globe turnip.

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